UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

THE INFLUENCE OF GENDER IN THE PREVALENCE OF NEONATAL SEPSIS IN HORSES: A RETROSPECTIVE STUDY OF 85 CASES

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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To my parents and my brother,
who believed in me long before I did.

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Firstly, I would like to thank Dr Luís Lamas for all the help, guidance and advice he has provided throughout this past year. His continuous efforts have undoubtfully improved the university's equine hospital, and I am very grateful for the opportunity to work with him, and hope to continue to do so in the future.

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Abstract

THE INFLUENCE OF GENDER IN THE PREVALENCE OF NEONATAL SEPSIS IN HORSES: A RETROSPECTIVE STUDY OF 85 CASES

Sepsis remains a leading cause of death in foals, despite considerable advances in the management of critically ill neonates. Identification of populations at an increased risk of developing sepsis allows for early diagnosis and treatment, improving the patient's outcome. Several factors have been previously associated with higher risks of sepsis. Although male gender has been associated with a higher prevalence of neonatal sepsis in mankind, gender predisposition has never been investigated in horses.

All foals 28-days-old or younger, admitted to Anglesey Lodge Equine Hospital in Ireland, between 2008 and 2016, diagnosed with sepsis and with their gender reported in their medical records, were included in this study. A group of foals without sepsis, admitted to the hospital during the same period, was used as the control group. Despite an apparently higher percentage of males in the sepsis group, analysis of gender distribution showed no significant differences between the two groups. Yet, interestingly, there was a significantly higher mortality rate in males with sepsis when compared to their female counterparts ($p<0.01$). These results indicate that gender may have an influence in the clinical evolution of a septic process in foals, but further investigations into the reasons of these findings are now necessary.

Keywords: sepsis; prevalence; foal; outcome; gender; male.

Resumo

A INFLUÊNCIA DO GÉNERO NA PREVALÊNCIA DE SÉPSIS NEONATAL EM CAVALOS: UM ESTUDO RETROSPETIVO DE 85 CASOS

A sépsis permanece uma das principais causas de morte em poldros, apesar dos avanços médicos no cuidado intensivo de neonatos. Identificar populações em risco permite um diagnóstico e tratamento precoces, melhorando o desfecho dos casos clínicos. Diversos fatores têm sido identificados como fatores de risco para o desenvolvimento de sépsis. Apesar do género masculino estar associado a uma maior incidência de sépsis neonatal no ser humano, esta associação nunca foi investigada em cavalos.

Todos os poldros com menos de 28 dias, recebidos no Anglesey Lodge Equine Hospital na Irlanda, entre 2008 e 2016, com diagnóstico de sépsis e com informação disponível acerca do seu género, foram incluídos neste estudo. Um grupo de poldros sem sépsis, recebidos no hospital durante o mesmo período, foi usado como controlo. Apesar da percentagem de machos aparentar ser maior no grupo de animais com sépsis, não foram encontradas diferenças significativas entre grupos. Notavelmente, a taxa de mortalidade dentro do grupo com sépsis foi mais elevada para os machos do que para as fêmeas ($p<0.01$). Os resultados indicam que o género poderá influenciar a evolução clínica da sépsis em poldros, mas serão necessários mais estudos para investigar possíveis explicações para as diferenças encontradas.

Palavras-chave: sépsis; prevalência; poldro; desfecho; género; macho.

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α – Alfa
β – Beta
κ – Kappa
$ – Dollar sign
ºC – Degrees Celsius
% – Percentage
ACTH – Adrenocorticotropic hormone
APTT – Activated partial thromboplastin time
CARS – Compensatory anti-inflammatory response syndrome
CD14 – Cluster of differentiation 14
CV – Coefficient of variation
d – Days
DAMPs – Damage-associated molecular patterns
DNA – deoxyribonucleic acid
EHV-1 – Equine herpesvirus type 1
FPT – Failure of passive transfer
g/dL – grams per decilitre
g/L – grams per litre
IFN – Interferon
Ig – Immunoglobulin
IL – Interleukin
IV – Intravenous
K.C. – Kevin Corley
L – Litre
LBP – Lipopolysaccharide binding protein
LPS – Lipopolysaccharide
MARS – Mixed anti-inflammatory response syndrome
mg/dL– Miligrams per decilitre
mL/kg – Mililiters per kilogram
mmHg – Milimeter of mercury
mmol – Milimoles
mmol/l – Milimoles per litre
mo – Months
MODS – Multiorgan dysfunction syndrome
n – Sample size
NF – Nuclear factor

NICU – Neonatal intensive care unit

PAMPs – Pathogen-associated molecular patterns

PCV – Packed cell volume

PT – Prothrombin time

pH – Potential of hydrogen

PRRs – Pattern recognition receptors

RNA – Ribonucleic acid

s– second

SAA – Serum Amyloid A

SCID – Severe combined immunodeficiency

s.d. – Standard deviation

SIRS – Systemic inflammatory response syndrome

TLRs – Toll-like receptors

TNF – Tumour necrosis factor

vs. – versus

WBC – White blood cell count

y – Years

CURRICULAR TRAINING REPORT

As part of the Integrated Masters Degree on Veterinary Medicine from the Faculty of Veterinary Medicine of the University of Lisbon, I completed two traineeships with a total duration of five months.

The first training period was spent in Anglesey Lodge Equine Hospital in Ireland, under the supervision of Dr Kevin Corley, and had a duration of three months. During that time, I had the opportunity to assist to a wide variety of cases in different areas of equine medicine, including internal medicine, surgery, orthopaedics, reproduction and neonatal care. I worked mostly with Thoroughbred racehorses, and was exposed to the most common conditions associated with horseracing. I helped with daily hospital procedures and supported the in-patient care, including daily general physical examinations, medications and treatments. I helped with ambulatory work and out-of-hours' cover when required. I helped with complementary exams such as gastroscopy, airway endoscopy, overground dynamic endoscopy, ultrasound and radiography. I also observed many advanced surgical procedures, including orthopaedic procedures (arthroscopy, fracture repair, placement and removal of transphyseal screws in foals, surgical wound cleaning and debridement), upper airway surgery (ventriculocordectomy, surgical advancement of the larynx, prosthetic laryngoplasty, soft palate cautery), laser-assisted procedures (transendoscopic laser resection of the aryepiglottic folds, laryngeal mass removal), soft tissue procedures (colic surgery), urogenital surgery (orchiectomy of undescended testicles, reconstruction of the perineal region in mares) and sinus surgery. I helped with lameness investigations, assisting with diagnostic imaging, and I also helped with specific treatments, such as injection of platelet-rich plasma, intra-articular injection of corticosteroids and mesotherapy. I also participated in cases discussions and presented scientific papers every other week during the hospital's journal club. At the end of the training period, I had the opportunity to work with Dr Corley on a case report of a thoroughbred national hunter presenting with vascular air embolism due to a suspected broncho-bronchial fistula. The report is currently being edited by Dr Corley, to be sent to Equine Veterinary Education.

The second period was spent at the Equine Surgery and Emergency Service of the Faculty of Veterinary Medicine of the University of Lisbon in Portugal, under the supervision of Dr Luís Lamas, and had the duration of two months. I was responsible for daily physical examinations and medication, and I supervised the volunteer students whenever they performed those tasks. I observed advanced surgical procedures such as arthroscopy, colic surgery and orchiectomy of undescended testicles, and helped with anaesthetic procedures such as induction, intubation and general monitoring of anaesthesia. I also helped with lameness investigation and diagnostic imaging, such as ultrasound and radiography. I participated in case discussions and treatment planning, and helped writing reports and discharge instructions. I also helped with instrument cleaning and sterilization.

As an extracurricular activity, I did a two-week externship at Cotts Equine Hospital in Wales. I had the opportunity to follow the interns' work closely, helping them with daily checks and medications during the day, and out-of-hours' work when required. I helped with numerous lameness investigations, dental procedures and appointments for measurements for The Joint Measurement Board. I was able to observe advanced surgical procedures such as arthroscopies and I also assisted to some physiotherapy sessions and followed the work of a remedial farrier.

The present dissertation was elaborated at the end of these training periods, using medical records from Anglesey Lodge Equine Hospital, kindly provided by Dr Kevin Corley.

I. INTRODUCTION

More than twenty years ago, sepsis was determined as the major cause of death in foals less than seven days old (Cohen, 1994). With the increasing number of neonatal intensive care units (NICUs) and the considerable advances that have been made into the diagnosis and medical management of critical ill neonates (Gayle, Cohen & Chaffin,1998), mortality rate from sepsis related diseases (such as pneumonia, enterocolitis and umbilical infections) has decreased (Galvin & Corley, 2010). However, sepsis remains a significant cause of death and is also the most important comorbidity of other neonatal diseases (Palmer, 2014).

Prompt identification and treatment of foals with sepsis is vital to ensure a positive outcome. A long duration of clinical signs prior to admission significantly decreases the likelihood of survival (Gayle *et al*., 1998), and even with appropriate treatment foals can deteriorate rapidly (Sanchez, 2005). Additionally, sepsis results in major economic losses due to high hospitalisation costs. A study by Gayle *et al*. (1998) in a university referral hospital in the United States reported the costs of hospitalisation of a critically-ill foal in the NICU to range from US$250 to >$1000 per day. Identification of populations of foals that are at an increased risk of developing sepsis and their prognosis for survival is therefore crucial to help owners making informed, evidence-based decisions regarding treatment options for seriously ill foals.

1.1. Equine neonatal sepsis

1.1.1. Evolution of concepts in human medicine

With its origin in the ancient Greek, “sepsis” is an ever-evolving concept that was initially used in the Hippocratic books as a term to describe dangerous and odorous biological breakdown, close to the current notion of “putrefaction” (Majno, 1991). It was only in the 1600s-1700s that Koch, Pasteur, Semmelweis and Lister, along with some other founders of modern microbiology and medicine, began to realise the link between bacteria and sepsis, changing our current understanding of this concept (Vincent & Abraham, 2006).

Prior to 1990, there was some uncertainty involving the terminology used to describe sepsis and its sequelae, and the lack of precise criteria made it difficult to assess the severity of this disorder (Bone, Sibbald & Sprung, 1992). With the goal of providing a conceptual and practical framework for the definition of sepsis in humans, the American College of Chest Physicians and the Society of Critical Care Medicine convened a “Consensus Conference” in 1991. Broad definitions for sepsis were proposed and the concept of systemic inflammatory response syndrome (SIRS) was first introduced. Definitions for septic shock, hypotension and multiple organ dysfunction syndrome were offered, and detailed physiologic parameters by which patients should be categorised were also proposed. This improved early bedside detection and

therefore early therapeutic intervention. It ultimately allowed the standardisation of research protocols and enhanced the application of information derived from clinical studies (Bone *et al*., 1992).

However, some clinicians believed the definition of sepsis was unclear, and that data regarding its pathophysiology should be updated. Acknowledging these opinions, the Society of Critical Care Medicine, the European Society of Intensive Care Medicine, the American College of Chest Physicians, the American Thoracic Society and the Surgical Infection Society held a new consensus in 2001. Definitions of sepsis and its related conditions were revisited, with the intention of increasing their accuracy, reliability and clinical utility. However, no evidence supported an alteration to definitions and changes consisted only on the expansion of the list of signs and symptoms for diagnosing infected patients with sepsis (Levy *et al*., 2003). Although some paediatric-specific diagnostic criteria were also introduced there was still little guidance in the literature for the definition of paediatric sepsis. Experts recognised that the physiological and laboratory variables used to defined SIRS in adults should be adjusted according to the developmental stages of children. For that reason, an International Pediatric Sepsis Consensus Conference took place in 2002, creating consensus definitions adjusted accordingly with the patients' age (Goldstein *et al*., 2005).

After the 2001 Consensus Conference, considerable advances were made into the pathobiology, management and epidemiology of sepsis, which once again suggested a need for an update of concepts (Vincent, Opal, Marshall & Tracey, 2013). The European Society of Intensive Care Medicine and the Society of Critical Care Medicine convened a third international consensus and a new definition for sepsis was proposed. Previously described as a systemic inflammatory response to infection, sepsis should now be regarded as a life-threatening organ dysfunction caused by a dysregulated host response to infection. This difference was justified by the current understanding that SIRS may simply reflect inappropriate host response while sepsis involves organ dysfunction, indicating a slightly more complex pathobiology (Singer *et al*., 2016).

Even though a lot of effort has been put on understanding this disorder, sepsis already proved to be a very complex process and defining it remains a work in progress. Practitioners are recommended to adopt the most recent definitions but should be aware of the possibility of future iterations.

1.1.2. Definitions for equine medicine

As far as equine medicine is concerned, the challenges faced were very similar to human medicine. For a long time, the terms sepsis, septicaemia, bacteraemia, septic syndrome and septic shock had varying definitions and were sometimes applied interchangeably, compromising the interpretation of available data (Palmer, 2014). No consensus conferences have been held for veterinary medicine and that was why, in search for accuracy and consistency among practitioners, veterinary specialists have extrapolated the definition of sepsis and its sequelae from the 1991 Consensus Conference (Corley, McKenzie, Amoroso & Furr, 2000; Corley, Donaldson & Furr, 2005; Taylor, 2015). The following paragraphs describe the sepsis related terminology and abbreviations that are currently in use.

The concept of systemic inflammatory response syndrome (SIRS) is described as a complex pathophysiological inflammatory response which results in at least two of the following clinical manifestations: hyperthermia or hypothermia; tachycardia; tachypnoea or hyperventilation; and leucopenia, leucocytosis or relative increase of circulating immature or band neutrophils (Table 1) (Sanchez, 2005; Taylor, 2015). SIRS can be initiated by a wide variety of insults, either infectious or non-infectious. Non-infectious insults may include trauma, extensive burns, sterile inflammatory processes such as pancreatitis, ischemia, haemorrhagic shock, surgery, among others. Infectious agents such as bacteria, fungi, viruses and parasites may also initiate SIRS, which is referred to as sepsis (Taylor, 2015). Figure 1 illustrates the relationship between SIRS, sepsis and infection for better comprehension.

Figure 1: Illustration of the relationship between SIRS, sepsis and infection, modified from McKenzie & Furr, 2001. Infection is not always associated with sepsis, and SIRS can occur without infection. It is only when SIRS occurs in association with infection that the response may be termed sepsis.

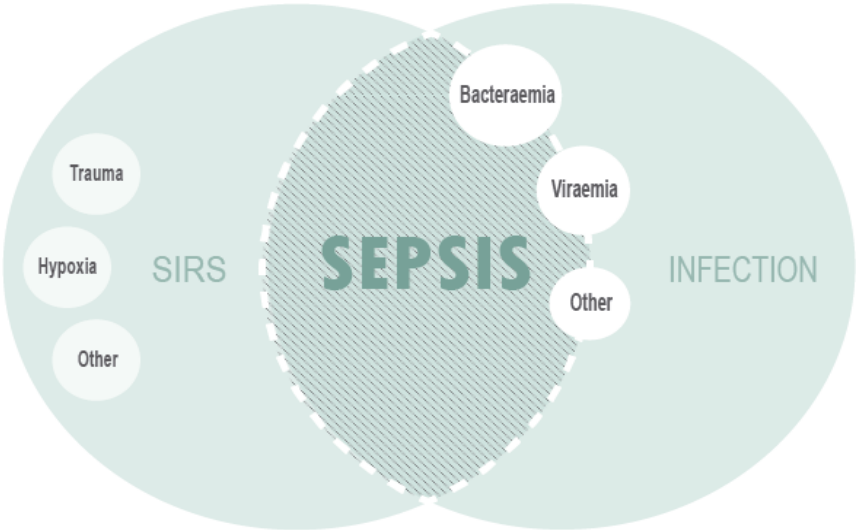


Table 1: Definition of SIRS and criteria used for foals, from Corley *et al.*, 2005.

Term	Definition	
Systemic inflammatory response syndrome (SIRS)	Systemic inflammatory response to a multitude of insults, manifested by at least 2 of the following conditions:	
	Temperature (rectal)	> 39.2ºC or < 37.2ºC
	Heart rate	> 120 beats/min
	Respiratory rate	> 30 breaths/min
	White cell count	> 12.5×10^9/L or < 4×10^9/L
	Band neutrophils	> 10%

If SIRS leads to severe hypotension not responsive to intravenous fluid therapy, it is termed shock. If this progresses to a state of hypoperfusion and organ dysfunction, and if homeostasis cannot be maintained without intervention, it is designated multiple organ dysfunction syndrome (MODS) (McKenzie & Furr, 2001). Manifestations of MODS in the horse can include laminitis, coagulopathy and gastrointestinal, hepatic, renal, cardiovascular or pulmonary dysfunction (Roy, 2004; Sanchez, 2005).

When an inflammatory response is developing the body's natural response is to counterbalance with an anti-inflammatory response. However, this might also become too pronounced, leading to a syndrome of immune suppression called compensatory anti-inflammatory response syndrome (CARS) (Roy, 2004; Taylor, 2015). Patients may alternate between episodes of SIRS and CARS, which is referred to as mixed anti-inflammatory response syndrome (MARS) (Roy, 2004).

As previously stated, sepsis is defined as a systemic inflammatory response to infection. When sepsis is associated with organ dysfunction, hypotension or hypoperfusion it is designated severe sepsis (McKenzie & Furr, 2001; Sanchez, 2005). If a patient with severe sepsis is hypotensive despite adequate fluid resuscitation the term septic shock is used (Sanchez, 2005; Taylor, 2015).

The term septicaemia is used in some papers to describe bacterial infection in the blood in conjunction with SIRS. However, its elimination from current usage has been suggested as it does not adequately describe the entire spectrum of pathogenic organisms that can be involved in a septic process (Taylor, 2015).

The term bacteraemia can be used if bacteria are present in the bloodstream (Taylor, 2015).

The term endotoxaemia is also commonly used and describes the presence of endotoxins such as gram negative bacterial lipopolysaccharide (LPS) within the blood. LPS is a component of the outer cell membrane of gram negative bacteria that is released during multiplication or bacteriolysis. By definition, LPS in the blood is not considered an "infection", but given the similar proinflammatory response endotoxaemia is considered a subset of sepsis (Taylor, 2015).

Even though these definitions have been used in most scientific papers in the last years, a widely-accepted definition of SIRS in foals has not been established yet. A recent paper by Wong and Wilkins (2015) emphasised the importance of using age-specific vital parameters, as well as clinicopathological parameters, when establishing criteria for SIRS in foals. The authors stated that, like in children, tachycardia and tachypnoea are common presenting symptoms of many disease processes in foals and lack specificity for SIRS. Additionally, they proposed that blood glucose and lactate levels should be included as criteria for equine sepsis, since they have both been significantly associated with SIRS and sepsis (Corley *et al*., 2005; Hollis *et al*., 2008). Based on this, the authors suggested a new definition for SIRS and modified criteria for foals (Wong & Wilkins, 2015). The proposed definition for SIRS implies a systemic inflammatory response to an insult, manifested by the presence of at least 3 of the following parameters, 1 of each must be abnormal temperature or leucocyte count: hyperthermia or hypothermia; tachycardia; tachypnoea; leucocytosis, leucopenia or an increase in band neutrophils; increase in blood lactate level; and a decrease in blood glucose level (Wong & Wilkins, 2015). The proposed criteria for foals are presented in Table 2.

Table 2: Modified criteria for SIRS in foals, proposed by Wong and Wilkins, 2015.

Parameter	Newborn foal (birth to 3d age)	Neonatal foal (4-14d age)	Juvenile foal (15d to 6 mo)	Weanling foal (7mo to 1y)
Hyperthermia or hypothermia (rectal temperature)	>39.2ºC or <37.2ºC	>39.2ºC or <37.2ºC	>39.2ºC or <37.2ºC	>39.2ºC or <37.2ºC
Tachycardia (beats/min)	>115	>120	>96	>60
Tachypnoea (beats/min)	>56	>56	>44	>20
Leucocytosis (×10^3), leucopenia (×10^3), or >5% band neutrophils	>14.4 or <6.9	>12.5 or <4.0	>12.5 or <4.0	>12.5 or <4.0
Venous blood lactate level (mmol/L)	>5.0	>2.5	>2.5	>2.5
Venous blood glucose level (mg/dL)	<50	<50	<50	<50

1.1.3. Overview of the pathophysiology of sepsis

Sepsis generally begins with a physical or immunological disruption of integrity of the host's surface barrier allowing entry of a pathogen, usually bacteria (Lever & Mackenzie, 2007). As a response to bacterial invasion, phagocytic cells and defensive molecules move from the bloodstream to the site of infection, soon activating the host's innate and adaptive immune systems (McKenzie & Furr, 2001; Wong & Wilkins, 2015). The adaptive immune system uses T and B lymphocytes to generate receptors that are specific for the antigens they encounter. They help neutralise and destroy infected cells and allow the production of memory T and B cells, granting a more efficient and rapid response in case of re-exposure to the same pathogen. However, this response can take several days to complete, which would leave the body defenceless if it were not for the innate immune response (Roy, 2004). The innate immune system recognises invading organisms through the use of pattern recognition receptors (PRRs) that detect specific pathogen-associated molecular patterns (PAMPs) of invading organisms and damage-associated molecular patterns (DAMPs). PAMPs are essential for microbial survival or virulence, so patterns have been conserved through evolution, often being shared between a broad range of organisms (Roy, 2004). Examples of PAMPs include: lipoteichoic acid, lipopeptides and peptidoglycan from gram-positive bacteria; LPS from gram-negative bacteria; unmethylated C-phosphage-G DNA from gram-positive and negative bacteria; flagellin from bacterial flagellum; and mycobacterial products (Werners & Bryant, 2012; Wong & Wilkins, 2015). DAMPs can be passively released from dead non-physiological cells or be actively secreted as alarm signals by necrotic and physiologically stressed cells. Examples of DAMPs include histones, glycoproteins, heat shock proteins, high-mobility group box protein-1, end products of advanced glycation, and extracellular RNA and DNA (Wong & Wilkins, 2015).

The host's PRRs can be transmitted throughout generations, using years of natural selection for survival advantage. For that reason, the immune system is ready to work from the moment it recognises invading pathogens, allowing time for adaptive response to initiate (Roy, 2004). There are three functional categories of PRRs: secreted PRRs, that act as opsonins and help in the recognition by the complement system and in phagocytosis; endocytic PRRs, that are present on the surface of phagocytic cells and bind to microbial components, mediating pathogen uptake and delivery to the endosome; and signalling PRRs, that bind to PAMPs and activate signal transduction pathways, promoting the transcription of numerous genes that are important for immunity (Roy, 2004). Each PRR recognises specific ligands and can be located on the cell surface, endoplasmic reticulum, endosomes, lysosomes or the cytosol. Examples of PRRs include toll-like receptors (TLRs), lectin receptors, retinoic acid inducible gene I-like receptors and nucleotide-binding oligomerisation domain (NOD)-like receptors (NLRs) (Werners & Bryant, 2012).

Because of the high frequency of Gram-negative infections (explained in more detail in section *1.1.4. Infectious agents*), endotoxaemia is a common sequela in foals, making the identification of an LPS receptor of great importance. It was known that, once in circulation, LPS binds to a LPS-binding protein (LBP), and this LPS-LBP complex later binds to a receptor on the surface of the mononuclear phagocyte referred to as mCD14. Yet, there was no knowledge of an intracellular connector that led to cellular activation and this uncertainty was only solved by the discovery of TLRs (Cohen, 2002; McKenzie & Furr, 2001). Toll-like receptors are the best characterised PRRs, and are viewed as important activators of the innate immune system (Roy, 2004, Werners & Bryant, 2012). The structure and alignment of TLRs' components determine which and how ligands bind to them. Of the 10 identified equine TLRs, 4 are known to bind to bacteria: TLR4, which recognises lipid A component from LPS; TLR2, that recognises Gram-positive bacteria and mycobacterial products; TLR5, that recognises flagellin; and TLR9, which recognises unmethylated C-phosphage-G DNA from bacteria and virus (Werners & Bryant, 2012).

Activation of PRRs triggers, among a series of other intracellular events, the activation of nuclear factor-κβ (NF-κβ), that regulates the transcription of several genes involved in the host response to infection, including proinflammatory cytokines (Roy, 2004; Werners & Bryant, 2012; Wong & Wilkins, 2015). Pro-inflammatory cytokines are synthesised by mononuclear phagocytes and include tumour necrosis factor (TNF-α) and interleukin (IL) 1 (McKenzie & Furr, 2001; Roy, 2004; Wong & Wilkins, 2015). TNF-α and IL-1 act synergistically and produce most of the clinical signs associated with sepsis including fever, depression, anorexia, tachycardia and tachypnoea. They induce the transcription of genes that encode other molecules involved in the inflammatory process including other cytokines and chemokines such as IL-2, IL-6, IL-8, IL-12 and interferon (IFN), phospholipase A_2, cyclooxygenase-2, inducible nitric oxide and adhesion molecules (Roy, 2004; Wong & Wilkins, 2015). They also induce the release of some acute phase proteins from the liver such as fibrinogen, C-reactive protein, mannose-binding lectin, complement, serum amyloid A, haptoglobin and vasoactive amines (Taylor, 2015). Several mediators are also produced including platelet-activating factor, prostaglandin E_2 (PGE_2) and leukotrienes (Roy, 2004).

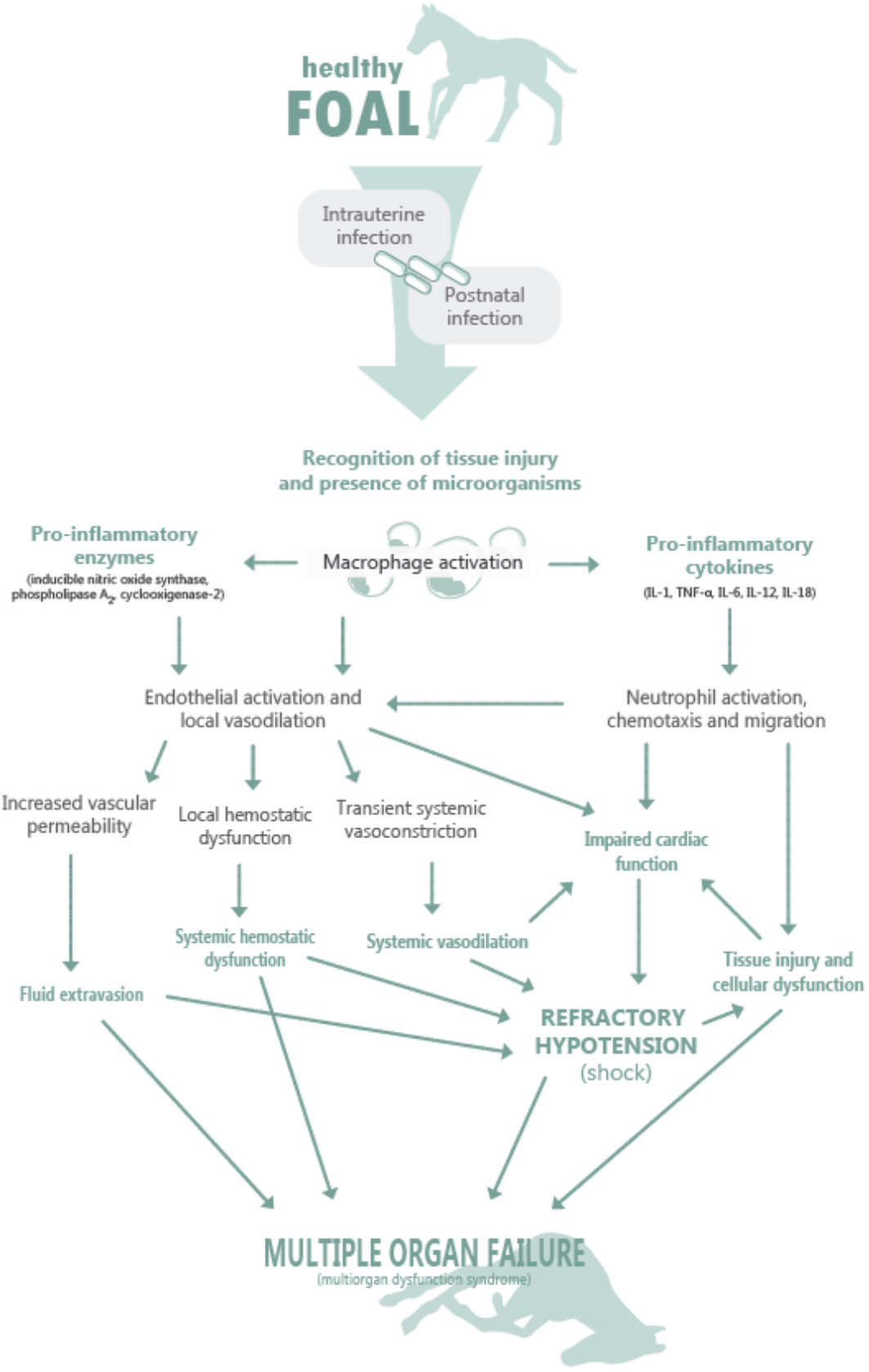
Pro-inflammatory cytokines are functionally related to the coagulation cascade, having a procoagulant effect. Concurrent activation of inflammation and coagulation may be effective in controlling localised infection but if the insult exceeds the host's ability to contain the inflammatory response locally, systemic inflammation ensues (Opal, 2000; Wong & Wilkins, 2015).

There are different mechanisms that contribute to the dysregulation of coagulation. Some cytokines promote the expression of tissue factor on the surface of endothelial cells and monocytes. Tissue factor, also referred to as thromboplastin, proceeds to activate the extrinsic coagulation cascade, in which prothrombin is converted to thrombin and fibrinogen turns into

fibrin. Concurrently, normal fibrinolytic mechanisms are impaired. The end result is increased production and reduced removal of fibrin, which leads to formation and deposition of fibrin clots. If fibrin cloths reach small blood vessels, tissue perfusion becomes inadequate and organ failure develops (Cohen, 2002; Opal, 2000; Roy, 2004, Wong & Wilkins, 2015). Additionally, cytokines promote endothelial dysfunction and the endothelium, instead of having an anticoagulant effect, becomes a procoagulant surface, with cells expressing adhesion molecules and vasoactive compounds (Roy, 2004). Activated neutrophils adhere to the endothelium and release damaging oxidants, phospholipases and proteases, that cause additional endothelial damage and increase microvascular permeability (Jacobi, 2002; Wong & Wilkins, 2015). Enhanced permeability and vasodilation may result in leakage of fluid form the vasculature and be clinically associated with hypotension, haemoconcentration and global oedema. When homeostasis and organ function are compromised to a point they cannot be maintain without intervention, MODS ensues (Figure 2) (Wong & Wilkins, 2015).

In an attempt to counterbalance the proinflammatory response and to minimise self-induced tissue damage, the organism activates several anti-inflammatory mechanisms. These include the production of anti-inflammatory molecules such as IL-4, IL-5, IL-10, IL-11, IL-13, transforming growth factor-β (TGF-β) and TNF and IL-1 receptor antagonists. It also involves changes in metabolic activity such as the increase of glucocorticoid production and release of sympathetic mediators (Cohen, 2002; Roy, 2004; Wong & Wilkins, 2015). If the anti-inflammatory pathway is overstimulated, a state of immunosuppression (CARS) develops. CARS has been observed in some patients with sepsis and is responsible for an increased expression of immunosuppressive cytokines and a decrease production of the proinflammatory ones. It is also associated with leukocyte anergy, with T-cell hyporesponsiveness, lymphocytopenia and decreased antigen presentation by monocytes (Wong & Wilkins, 2015). In summary, an excessive anti-inflammatory response may be as harmful as an exaggerated proinflammatory response (Jacobi, 2002) and some authors consider it a potential mediator of sepsis (Cohen, 2002).

Figure 2: Illustration of the pathogenic networks in sepsis, showing how microbial components simultaneously activate multiple parallel cascades that result in multiple organ dysfunction. Modified from McKenzie & Furr, 2001.



1.1.4. Infectious agents

Virtually any infectious organism can lead to sepsis (Remick, 2007), yet bacteria and their products are the most frequent initiators of this disorder in horses (Roy, 2004). Gram-negative bacteria are the most prevalent organisms isolated in bacteraemic neonatal foals, with *Escherichia coli* being consistently reported as the most frequent isolate (Corley, Pearce, Magdesian & Wilson, 2007; Russell, Axon, Blishen & Begg, 2008; Theelen, Wilson, Edma, Magdesian & Kass, 2014). This enterobacterium is part of the horse's normal gastrointestinal flora and is possibly acquired by the foal during nursing, due to faecal contamination of the mare's udder (Corley *et al*., 2007). Other Gram-negative isolates described in foals with sepsis include *Actinobacillus spp.*, *Klebesiella spp.*, *Enterobacter spp*., *Salmonella spp*., *Proteus spp*., *Pseudomonas spp*. and *Acinetobacter spp.* (Corley *et a*l., 2007; Theelen *et a*l., 2014). Still, the percentage of Gram-positive isolates has been significantly rising over the last few years (Russell *et al*., 2008; Theelen *et al*., 2014). Gram-positive isolates may include *Streptococcus spp.*, *Enterococcus spp.*, *Staphylococcus spp.* and *Corynebacterium spp.* (Corley *et al*., 2007; Theelen *et al.,* 2014). This increase in Gram-positive isolates could eventually be explained by the widespread use of antimicrobial drugs, which favoured the selection of resistant species. Among them is *Enterococcus spp.,* relevant not only due to its unpredictable susceptibility patterns, but also due to its ability to act as donor of antimicrobial resistance genes to other bacteria (Theelen *et al*., 2014).

The relative frequency of isolation of each bacterial species tends to differ among studies. Bacterial populations can differ due to geographic and climatic variations, but can also be indirectly selected through different management practices on breeding farms. Differences in the patterns of antimicrobial use for both prophylaxis and treatment also have an impact on bacterial selection. Variation in the criteria of inclusion of foals in the studies is also significant, and often includes differences in age ranges, collection of samples and laboratory techniques (Theelen *et al*., 2014).

Most infections are caused by a single organism, but mixed infections are also described. Mixed infections can consist of Gram-positive or Gram-negative microorganisms, a mixture of both or any of these in combination with non-bacterial infectious agents. Other infectious organisms that have been associated with sepsis in equine neonates include equine herpes virus type 1 (EHV-1) (Murray *et al*., 1998), *Candida albicans* (Reilly & Palmer, 1994) and *Histoplasma capsulatum* (Rezabeck *et a*l., 1993).

1.1.5. Predisposing factors and sources of infection

The different events that predispose a foal to sepsis can be grouped into two main categories: prenatal or maternal factors, and postnatal factors (Sanchez, 2005).

Maternal factors include premature placental separation, placentitis and alterations in gestational length, particularly prematurity. Placentitis may be triggered by ascendant infections and subsequently lead to placental separation, foetal infection and premature delivery (McKenzie & Furr, 2001; Sanchez, 2005). Premature foals are relatively immune deficient, which in itself predisposes them to infections. Dystocia may too be a risk factor, as it can result in trauma to the foal. Some other forms of illness in the mare such as colic may also predispose a foal to sepsis, as foals *in utero* show less ability to respond immunologically to the insults they encounter (Bedenice, n.d.). Prolonged transport of the pregnant mare may also be a risk factor (McKenzie & Furr, 2001).

Of the postnatal events, the major risk factor for foals is failure to receive adequate quantity and quality of colostral antibodies at the appropriate time, referred to as failure of passive transfer (FPT). Because the foal is relatively immune-naïve at the time of birth, deprivation of colostrum leads to an increased susceptibility to infection, as immunoglobulin levels remain low and neutrophil function remains impaired (Robinson *et al*., 1993; Sanchez, 2005; Lester & Axon, 2015). Infection can occur through different portals of entry, with the gastrointestinal tract being the most significant. Unsanitary conditions and high stock density can result in an increased bacterial load to the gastrointestinal tract, exposing the foals to disease. Other relevant routes of infection include the umbilicus, wounds and the respiratory tract (McKenzie & Furr, 2001; Sanchez, 2005).

It is possible that genetic factors may also play a role, as a foal's susceptibility to infection may vary according to his genome. It seems reasonable that with the development of comparative genomics, some genetic polymorphisms that affect the foal's response to infection may be found in the future (Roy, 2004).

1.1.6. Clinical signs and diagnosis

Identification of foals with sepsis is of utmost importance to ensure appropriate management, since delays in treatment can compromise the foal's survival (Brewer & Koterba, 1988; Gayle *et al*., 1998). Identification of the non-infected foal is equally important to avoid unnecessary expenses, development of antibiotic resistances and side-effects (Brewer & Koterba, 1988; Corley & Furr, 2003). The diagnosis of sepsis is essentially presumptive, based upon the overall clinical impression obtained from a combination of clinical variables (Stewart *et al*., 2002). Medical history, physical examination and laboratory findings are the foundations for diagnosis, and are described in more detail in the following sections.

1.1.6.1. Medical history

A complete medical history is important to appreciate the extent of exposure of the neonate to any factors that increase the likelihood of sepsis. The different events that may predispose a foal to infection have already been discussed in the previous section (*1.1.5 Predisposing factors and sources of infection*).

1.1.6.2. Physical examination findings

Severe sepsis is felt to be easily recognisable by most practitioners (Roy, 2004). However, early clinical signs in foals can be vague and extremely nonspecific, and are sometimes overlooked by unexperienced observers (Roy, 2004; Sanchez, 2005). Early signs of sepsis in neonates frequently include slight depression, anorexia, decreased borborygmi, lack of daily weight gain, abdominal discomfort and lethargy, that may progress to recumbence. Tachycardia and tachypnoea might be present and mucous membranes may appear hyperemic and with rapid capillary refill time. Rectal temperature may be normal or slightly increased. Additionally, foals can present with focal sites of infection, with diarrhoea being one of the most common early localising sites. Other signs may include joint effusion, uveitis, respiratory distress, patent urachus and omphalitis (Roy, 2004; Sanchez, 2005).

As time progresses, infection overwhelms the foals' immune system and its compensatory responses, resulting in septic shock. Foals become hypoglycaemic and dehydrated, and the cardiovascular system becomes compromised from hypovolemia. Mucous membranes become dark and injected and often display a toxic ring. Capillary refill time increases, pulse weakens, rectal temperature decreases and extremities become cold, all of these reflecting peripheral shutdown. If left untreated, this process invariably leads to death (Roy, 2004; Sanchez, 2005).

1.1.6.3. Laboratory findings

There is no specific biochemical or haematologic parameter that distinguishes sepsis from other neonatal diseases (Koterba, Brewer & Tarplee, 1984). However, there is a collection of laboratory findings that when interpreted in conjunction with medical history and clinical signs can be highly suggestive of a septic process. Table 3 summarises the most frequent findings in foals with sepsis.

Abnormal white blood cell count is common and usually consists of leucopenia, mainly characterised by neutropenia, with a degenerative left shift. However, some older foals may present leucocytosis with neutrophilia. There is usually evidence of neutrophil toxicity, with the presence of Doehle bodies, toxic granulation and vacuolisation and an increased number of band neutrophils (Koterba *et al*., 1984; Barton, Morris, Norton & Prasse, 1998).

Since a considerable number of infections may be caused by failure of passive transfer (Robinson *et al*., 1993), foals with sepsis can present low immunoglobulin levels. The main immunoglobulin present in the colostrum is gamma globulin (IgG) (Reed, Bayly & Sellon, 2010), and for that reason laboratory tests measure serum levels of IgG. Foals with sepsis may display levels of IgG inferior to 4 g/L (Lester & Axon, 2015).

Serum glucose concentrations are usually low, mainly due to decreased nursing (Koterba *et al*., 1984). Hypoglycaemia can also be present in cases of endotoxaemia, since endotoxins increase the peripheral uptake of glucose and decrease hepatic gluconeogenesis (Sanchez, 2005).

Arterial blood analysis usually reveals metabolic acidosis and increased lactate (Corley *et al*., 2005; Sanchez, 2005). Lactate values are usually greater for foals who present a positive blood culture. Arterial lactate has also been shown to have a good correlation with mean arterial pressure, being a good indicator of the foal's cardiovascular status (Corley *et al*., 2005).

As for arterial blood pressure, it may be reduced or normal; this is particular important because foals with an apparently normal blood pressure may still have profound cardiovascular disturbances (Corley, 2002). For that reason, markers of hypoperfusion such as hyperlactatemia, hyperaldosteronaemia and hypervasopressinaemia may be a better estimator of tissue perfusion in foals (Dembek *et al*., 2016).

Because the coagulation and fibrinolytic systems are usually abnormal in a septic process, prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and fibrin degradation products may be elevated, while antithrombin III may be decreased (Barton *et al*., 1998). Fibrinogen is also a nonspecific indicator of inflammation and is one of the most frequently measured acute phase proteins. However, it has as slow rise, which compromises its utility (Stoneham, Palmer, Cash & Rossdale, 2001). If measured shortly after birth, high fibrinogen levels may also be a sign of *in utero* infections (Koterba *et al*., 1984).

When foals reach a state of hypovolemia and pulmonary dysfunction, increases in packed cell volume (PCV) and arterial hypoxemia can be noted (Wilkins, 2009). Creatinine may also be

elevated, reflecting end organ dysfunction or hypoperfusion (Wilkins, 2009). Azotemia and hyperbilirubinemia may also be present (Sanchez, 2005). One study showed that foals with sepsis also have significantly lower calcium and higher phosphorus and parathyroid hormone concentrations than healthy foals (Kamr *et al*., 2015).

Table 3: Summary of the most common laboratory findings in foals with sepsis.

Parameters	Findings	Reference range
Haematology	Increased packed cell volume	32 – 46 % [a]
	Leucopenia or leucocytosis	4.9 – 11.7×10^9/L [a]
	Increased band neutrophils	< 0.2 ×10^9/L [b]
Biochemistry	Increased creatinine	1.2 – 4.2 mg/dL [a]
	Hyperbilirubinemia	1.3 – 4.5 mg/dL [a]
	Hypocalcemia	11.7 ± 2.0 mg/dL [a]
	Hyperphosphatemia	5.6 ± 1.8 mg/dL [a]
	Decreased IgG	> 8 g/L [c]
	Hypoglycaemia	121 – 233 mg/dL [a]
	Hyperlactataemia	0.6 -1.9 mmol/L [a]
Arterial blood analysis	Metabolic acidosis	pH 7.364 – 7.444 [a]
	Hypoxaemia	66.5 ± 2.3 mmHg [a]
	Normal or reduced mean arterial blood pressure	> 60 mmHg [a]

[a]Reference range for one-day old foals from Corley & Stephen, 2008; [b]Reference range from Koterba *et al*., 1984; [c]Reference range from Liepman *et al*., 2015 .

In recent years, measurement of biological laboratory markers or biomarkers has been gaining popularity (Taylor, 2015). Ideally, a marker should be sensitive enough to detect infection in patients with minimal host response and specific enough to discriminate infection from other stimuli that may induce a similar inflammatory response. It should be present early in the course of disease, be easily measured and of prognostic value (Palmer, 2014). Until the present moment, no marker has qualified the “ideal” marker. Some of the biomarkers that have been successfully established as markers of inflammation and infection in human patients have also been investigated for their use in horses. The results varied according to the different markers investigated. Adrenomedullin, C-reactive protein and procalcitonin, which are significantly increased in infants with sepsis showed no significant association with sepsis in neonatal foals (Pusterla, Magdesian, Mapes & Leutenegger, 2006; Toth, Slovis, Constable & Taylor, 2015; Zabrecky, Slovis, Constable & Taylor, 2015). Haptoglobin, an acute phase protein that has significantly higher concentrations in infants with sepsis, showed to have significantly lower concentrations in foals with sepsis (Zabrecky *et al*., 2015).

Increased levels of soluble CD14 and serum amyloid A were significantly associated with sepsis in foals. CD14 is a component expressed on the surface of equine monocytes and macrophages (mCD14) that works as a receptor for the LPS-LBP complex and associates it

with TLR4, resulting in the synthesis and release of pro-inflammatory cytokines. Soluble CD14 is produced by shedding or exocytosis of CD14 and has an anti-inflammatory role, inhibiting the binding of LPS to endothelial cells. In human patients, sCD14 is one of the biomarkers used to evaluate the infants' susceptibility and/or progression in neonatal sepsis. Plasma concentrations of sCD14 proved to be significantly greater in samples from foals with sepsis (Wagner, Ainsworth & Freer, 2013), proving its biomarker usefulness, however, further studies are still necessary. Serum amyloid A is an acute phase protein that has been identified as a very sensitive and rapidly responding marker of inflammation and infection in foals (Chavatte *et al*.,1991). More recent studies (Stoneham *et al*., 2001; Húlten & Demmers, 2002) showed that foals with bacterial infections had significantly higher SAA concentrations on admission when compared to nonbacterial diagnoses, confirming this assertion. These studies also showed that SAA concentrations were lower at discharge, supporting the impression that SAA decreases rapidly after the insult starts to resolve. SAA is therefore a useful biomarker for infection, and has been advocated for use in the diagnosis of equine neonatal sepsis (Stoneham *et al.*, 2001).

1.1.6.4. Blood culture

The gold standard for the diagnosis of sepsis is blood culture. It allows for identification of the causative organism and its antibiotic susceptibility, granting a directed antimicrobial therapy. However, relying exclusively on the results of a blood culture to diagnose sepsis and institute therapy is questionable. First, results cannot be obtained for at least 24 to 48 hours, which would significantly delay treatment (Taylor, 2015). Furthermore, despite being extremely specific, blood culture does not confer optimal sensitivity, which means foal with sepsis may present negative blood results (Brewer & Koterba, 1988; Sanchez, 2005; Wong & Wilkins, 2015). This may be due to previous antimicrobial therapy, low number of circulating bacteria, relatively low volume of blood tested or varying blood culture systems that may yield different results (Sanchez, 2005; Taylor, 2015). False-positive results may also occur. A recent study demonstrated that healthy foals undergo a transient state of bacteraemia early in the post-partum period (Hackett *et al*., 2015), which could eventually provide a false-positive blood culture. Cross-contamination can also produce false positive results. All this evidence highlights the importance of relying not only on laboratory findings, but also having a strong clinical suspicion supported by the presence of several signs of sepsis.

1.1.7. Sepsis scoring system

A need for a quick and accurate means of determining the likelihood of sepsis was one of the main reasons why in 1988 a weighted scoring system to predict sepsis was published (Brewer & Koterba, 1988). Based on the retrospective evaluation of 19 cases of foals less than 13-days-old, the authors chose parameters they felt were strongly associated with sepsis. These included historical data, objective positive clinical examinations, subjectively evaluated clinical findings and clinicopathological laboratory data (Table 4). Different point values (weightings) were assigned to the various parameters and a cut-off score of 12 was determined, with higher scores representing a higher likelihood of sepsis or focal infection. Statistical analysis showed that the correlation of the sepsis score with sepsis was far superior than any parameter assessed individually. Sensitivity, specificity, false positive and false negative percentages were calculated, and values obtained were respectively 93%, 86%, 11% and 8%. Because of the difficulties in obtaining blood gas and arterial oxygen analyses, the sepsis score was re-evaluated, and the variables “metabolic acidosis” and “arterial oxygen” were eliminated. A new cut-off point of 11 was determined by logistic regression. This modified score proved almost as sensitive as the original scoring system (92.8%), slightly more specific (87.5%) and with a slightly lower percentage of false positives (9.4%), but with a higher percentage of false negatives (9.7%). Since the selection of criteria for the sepsis scoring system was empirical, it was hypothesised that a computer-generated scoring system would be more sensitive and specific. A new system was proposed (Brewer, Koterba, Carter & Rowe, 1988), and proved to be slightly more specific (87.5%) and with a lower percentage of false positives (9.8%), but less sensitive (89.2%) and with a higher percentage of false negatives (13.8%).

A higher sensitivity means a higher accuracy in classifying a foal with sepsis, whereas a higher specificity means accurately classifying a foal without sepsis. Since failure to treat an animal with sepsis is of greater consequence that unnecessarily treating an animal without sepsis, priority is placed on the systems with higher sensitivity. For the same reason, a lower percentage of false negatives or missed foals with sepsis, is also desirable. When comparing the three systems, the original sepsis score and the modified score proved to be statistically superior, and so the use of the computer-generated score was not recommended (Brewer *et al*., 1988).

Since its publication the score was used to predict and define sepsis in different studies (Barton *et al*., 1998; Gayle *et al*., 1998; Robinson *et al*., 1993). Yet, some clinicians felt that when used in a population from a different geographical area it was not as accurate as described in the original study (Corley & Furr, 2003). Corley and Furr (2003) proposed to determine the system’s accuracy in a different referral centre, collecting information from all foals admitted to the hospital during a 4-year period. All eligible foals were classified in accordance to the original and the modified sepsis scores. Sepsis was confirmed by positive blood culture, culture of sites of suspected local infection, clinical course and/or *post mortem* examination, and this

information was then used to calculate sensitivity, specificity, positive predictive value and negative predictive value for both scores. Values obtained were respectively 67%, 76%, 84% and 55% for the modified score, and 72%, 70%, 84% and 35% for the original score. The sensitivity and specificity of both scores were only considered moderate, and unquestionably lower than those from the original studies. Negative predictive values were low, meaning that the percentage of false negatives was considerable and limiting the clinical utility of the scores. Neither the sepsis score nor the modified sepsis score were found suitable to define sepsis in clinical studies, unless first validated in the hospital where the study was to be performed (Corley & Furr, 2003).

Regardless of the results obtained in any of the referred scores, clinical judgement remains a critical component of case evaluation. A diagnosis of sepsis must rely on a strong clinical suspicion supported by the combined presence of several of the signs and laboratory findings, always bearing in mind the importance of early intervention (Brewer *et al*., 1988; Gayle *et al*., 1998; Corley & Furr, 2003; Vincent & Abraham, 2006; Palmer, 2014; Weber *et al*., 2015).

Table 4: Sepsis scoring system, proposed by Brewer and Koterba, 1988.

Information collected	Number of points to assign 4	3	2	1	0
I. Complete blood count					
1. Neutrophil count (not total WBC)		<2.0×10^9/l	2.0-4.0 or >12.0	8.0-12.0	Normal
2. Band neutrophil count		>2.0×10^9/l	0.05-0.20		<0.05
3. Doehle bodies, toxic, granulation, or vacuolization in neutrophillis	Marked	Moderate	Slight		None
4. Fibrinogen (g/l)			>6.0	4.1-6.0	≤4.0
II. Other laboratory data					
1. Hypoglycaemia			<2.7 mmoL/l	2.7-4.4	>4.4
2. Zinc sulphate turbidity test (g/l) [(a)]	<2.0	2.0-4.0	4.1-8.0		>8.0
3. Arterial oxygen		<40 Torr	40-50	51-70	>70
4. Metabolic acidosis				Yes	No
III. Clinical examination					
1. Petechiation or scleral injection not secondary to eye disease or trauma		Marked	Moderate	Mild	None
2. Fever			>38.9ºC	<37.8ºC	Normal
3. Hypotonia, coma, depression, convulsions			Marked	Mild	Normal
4. Anterior uveitis, diarrhoea, respiratory distress, swollen joints, open wounds		Yes			No
IV. Historical data					
2.1. Placentitis, vulvar discharge prior to delivery, dystocia, long transport of mare, mare sick, foal induced		Yes			No
2.2. Prematurity		<300 days	300-310	311-330	>330

[(a)] If a foal is older than 12h, compute the score using the ZST value from the lab. If it is less than 12h, give it +2 for ZST if it has a history of nursing what appeared to be good colostrum. Give the foal a +4 if it has not nursed or if in doubt.

1.1.8. Management and treatment

Treatment of sepsis aims to neutralise the causative organisms and counterbalance the exaggerated immune responses, while maintaining homeostasis (Dunkel & Corley, 2015). The most reliable methods of managing sepsis include infection control and cardiovascular, respiratory and nutritional support (Palmer, 2014).

Controlling infection relies mostly on appropriate antimicrobial therapy. Ideally, therapy should only be initiated when the results from blood culture become available and should be directed according to the pathogens' sensitivity. However, since delaying treatment decreases the likelihood of survival, therapy should be initiated as soon as there is a suspicion of infection. The initial therapy is empirical (Palmer, 2014) and broad-spectrum antimicrobials are preferred at this stage (Dunkel & Corley, 2014). Some clinicians rely on community-acquired and nosocomial isolates' sensitivities, along with patient-specific clues to direct their initial therapy (Palmer, 2014). If necessary, therapy can be changed when the results from the blood culture become available (Sanchez, 2005). In cases where a primary site of infection can be identified, directed local therapy is recommended (e.g. removal of catheter if this is the source, joint flush in cases of septic joint). Dosing antimicrobials should be based on the pharmacokinetics in the neonatal foal (Dunkel & Corley, 2014), yet some drugs may require adaptations due to varying host factors and pathogen characteristics (Palmer, 2014). Length of treatment may also vary according to each case. In the past, a return to normal concentration of acute phase proteins was used as the indicator to discontinue antimicrobial therapy; however, this may lead to overtreating, with adverse effects not only for the individual patient but for the general population. Currently, clinicians believe it is sensible to discontinue antimicrobial treatment 2 to 5 days after recovery of the initial insult and if there is no clinical evidence of infection (Dunkel & Johns, 2015). Close observation after discontinuing treatment is imperative.

Because endotoxins are frequently present in equine sepsis, targeted therapy against LPS has been developed. Polymyxin B is an antimicrobial drug known to act as a chelating agent, binding endotoxin and thus preventing activation of inflammation (Wilkins *et al*., 2009; Wong *et al*., 2013). Administration of IV polymyxin B has showed to attenuate clinical signs and clinicopathological derangements in neonatal foals with induced endotoxaemia, and may be beneficial as an adjuvant treatment in neonates with sepsis (Wong *et al*., 2013). However, caution is required when using this drug due to its nephrotoxic potential.

The use of equine plasma in the treatment of neonatal sepsis is common, and likely beneficial. A study by Peek *et al*. (2006) found that foals with sepsis that received a plasma rich in antiendotoxin antibodies had a higher survival rate to discharge compared to foals which received a conventional, commercially available hyperimmune plasma. However, due to the potential complications associated with administration of plasma or other blood derived products, large-scale controlled clinical trials are required before true evidence-based recommendations can be made (Wilkins, 2009).

Fluid therapy is essential in the management of critically ill neonates, especially in foals presenting with septic shock (Sanchez, 2005). However, the common use of an aggressive volume for resuscitation has been questioned, as it may lead to fluid overload and further disruptions in patients with sepsis (Palmer, 2014). Because sepsis is often a problem of reduced vascular capacitance rather than true hypovolemia, fluid resuscitation should be performed using boluses of fluids (20 mL/kg), as opposed to continuous infusion, along with vasopressors to restore vascular tone (Palmer, 2004; Palmer, 2014). The most commonly used drugs include dobutamine, dopamine, norepinephrine, epinephrine and vasopressin. Norepinephrine has showed to increase blood pressure in hypotensive neonatal foals that do not respond to fluid therapy and inotropes (Corley *et al*., 2000). Yet, there are still no studies comparing the different drugs, so the choice between them is mainly personal (Palmer, 2014). Foals in which these drugs are used should be closely monitored, and the need for further administration should be frequently reassessed (Dunkel & Corley, 2014; Palmer, 2014).

Improving haemodynamics will enhance oxygen delivery to tissues, but pulmonary transfer of oxygen should also be addressed. Ventilation allows reduction of the work of breathing and increases cardiovascular function, optimising gas transport (Palmer, 2014). Intranasal oxygen is recommended, and should be supplied via uni- or bilateral cannulas, in the lowest flow rate possible to prevent oxygen toxicity (Dunkel & Corley, 2015). Nutritional support is also important, and foals should be provided enteral nutrition through a nasogastric tube if they are unable to nurse. The only exceptions are cases of severe sepsis, in which enteric alimentation can result in abdominal distension, colic, diarrhoea and nasogastric reflux, and prolong the duration of disease. In such cases, energy requirements should be met by administering intravenous glucose or through parenteral nutrition (Dunkel & Corley, 2015). Glucose levels should be assed, as hyperglycaemia may be as harmful as hypoglycaemia. If hyperglycaemia occurs, insulin may be administered in a continuous rate infusion (Palmer, 2014).

Anti-inflammatory therapy has been considered a logical step in the treatment of sepsis (Wilkins, 2009), with flunixin meglumine being the more frequently used drug. The principle behind its use is the blockage of pathways that lead to systemic organ dysfunction, vascular collapse and death. However, these pathways may also be involved in protective roles and targeting them can be of no therapeutic value, or can even be detrimental (Palmer, 2014). Anti-inflammatory therapy can therefore be questioned, and more studies are needed before reaching any conclusions. The use of corticosteroids for inflammatory suppression has also been tried without success (Palmer, 2014). However, administration of low doses of cortisone were shown to reduce the pro-inflammatory response without compromising neutrophil function in *ex vivo* models of neonatal foals (Hart, Barton, Vandenplas & Hurley, 2011). Further studies should be developed to assess the clinical effects of low-dose hydrocortisone *in vivo*.

1.1.9. Prognostic indicators and outcome

The prognosis for survival to discharge of neonates with sepsis varies widely, with reported survival rates from as low as 10% (Hoffman *et al*., 1992) to as high as 71% (Raisis, Hodgson & Hodgson, 1996; Toth *et al*., 2014). Numbers have improved over time, with recent studies showing survival rates ranging mainly between 44 and 71% (Dunkel & Corley, 2015). Numerous factors may have contributed to this improvement including clinician's greater knowledge of neonatal diseases, development of NICUs, early referral of sick neonatal foals and improved awareness of neonatal illnesses by the owners (Axon, Palmer & Wilkins, 1999). However, the main reasons why sick foals are euthanised include not only poor prognosis for survival, but also financial limitations and expectations of a lower performance (Dembek, Hurcombe, Frazer, Morresey & Toribio, 2014). Using clinical information to predict the likelihood of survival and future performance is therefore of interest, as it may facilitate the decision-making process for owners (Dembek *et al*., 2014).

Studies in critically ill foals show that the presence of maternal disease during pregnancy (Furr, Tinker & Edens, 1997) and history of induced parturition (Gayle *et al*., 1998) are more frequently associated with death of neonates with sepsis. Survival is also influenced by the duration of clinical signs prior to admission, with foals that are admitted earlier being more likely to survive (Gayle *et al*., 1998). A decreased respiratory rate, a subnormal temperature and recumbency at admission have also been associated with a poorer prognosis (Furr *et al*., 1997; Gayle *et al*., 1998).

Because physical examination can only concede a limited amount of information, different laboratory parameters have been investigated as prognostic indicators. Leucopenia and neutropenia have both been associated with nonsurvival (Gayle *et al*., 1998). Leucopenia usually results from increased peripheral consumption of lymphocytes, reflecting a reduced ability to clear infection (Furr *et al*., 1997). Neutropenic foals are estimated to be 37 times more likely to die than foals with normal neutrophil counts (Gayle *et al*., 1998). Foals with hypoglycaemia, hypoalbuminaemia, acidemia, increased anion gap and venous hypoxemia at admission are too at an increased risk of dying (Hoffman, Staempfli & Willan, 1992; Furr *et al*., 1997; Gayle *et al*., 1998). Hyperlactatemia at admission has also been identified as a prognostic indicator of nonsurvival in critically ill foals (Corley *et al*., 2005; Wotman, Wilkins, Palmer & Boston, 2009). Sequential measurements of lactate concentrations may be useful in assessing response to treatment and prognosis over time, although this requires further investigation (Wotman *et al*., 2009). High fibrinogen concentration, low IgG concentration and low total red blood cell numbers at admission are also associated with a poor prognosis for survival (Peek *et al*., 2006). Other metabolic and endocrine derangements that have been associated with nonsurvival include high adrenocorticotropic hormone (ACTH) concentrations, high plasma cortisol levels, low ACTH/cortisol ratio, high levels of triglycerides, creatinine, urea and glutamate dehydrogenase (Armengou *et al*., 2013), hypovitaminosis D, hypocalcaemia,

hyperphosphataemia, higher parathyroid hormone concentrations (Kamr *et al*., 2015) and higher vasopressin concentrations (Borchers, Magdesian, Schenck & Kass, 2014).

In 2014, Dembek *et al*. published the first survival scoring system for equine neonates, meant to help clinicians predict the likelihood of survival of foals based on clinical information obtained on admission. The authors developed the system based on the retrospective evaluation of 339 cases of foals less than 4-days-old, and later validated it in a prospective study with 285 neonates. Thirty-seven variables were analysed, of which six were retained in the final model. Different weightings were then assigned to each parameter (Table 5). Foals with a total score ≥4 were predicted to survive and <4 were predicted to die. This classification showed a sensitivity of 96%, a specificity of 71%, a positive predictive value of 91% and a negative predictive value of 85%. The survival scoring system revealed a simple tool to help determining the likely prognosis for survival. Still, it should not be used in isolation to make decision regarding euthanasia nor should it replace clinical judgment (Dembek *et al*., 2014).

Table 5: Survival scoring system in hospitalised neonatal foals, proposed by Dembek *et al*. (2014).

	Number of points to assign		
Variables	**0**	**1**	**2**
Cold extremities	Yes		No
Prematurity (<320 days)	Yes	No	
≥ 2 inflammation/infection sites	Yes	No	
IgG (mg/gL)	<400	≥ 400	
Glucose (mg/dL)	<80	≥80	
WBC x (103/uL)	≤4	>4	

In terms of athletic performance of surviving neonates with history of sepsis, some studies have identified differences in performance-related parameters. One study showed that Thoroughbred and Standardbred horses that had been discharged from an intensive care unit had a lower percentage of starters when compared to the control group, but no differences in performance over a 2-year period were found (Axon *et al*., 1999). However, a second study showed that surviving foals were as likely to race or win as the controls, but did not earn as much money and had lower overall performances (Sanchez, Guigère & Lester, 2008). History of septic arthritis as a foal has also been associated with a decreased likelihood of winning races in Thoroughbred horses (Steel *et al*., 1999). Yet, no differences were found regarding sales price between survivors and controls (Corley and Corley, 2012).

These issues should be taken into consideration by clinicians when offering prognosis to owners. Still, the disparities in the conclusions obtained raise the need for further investigation, preferably in large scale studies.

1.1.10. Prevention

Given the potentially devastating outcomes associated with sepsis, prevention always outweighs treatment. The preventive strategies generally address risk factors for sepsis and therefore depend on the individual situation of each farm (Sanchez, 2005).

Mares should be followed throughout pregnancy and parturition, so that eventual complications such as placentitis or premature placental separation can be promptly identified and addressed. Maternal nutrition and health care, including prepartum vaccination and deworming, are also relevant and may help prevent *in utero* infections (Sanchez, 2005; Dunkel & Corley, 2015).

In a general manner, owners should maintain a clean environment, particularly during foaling. Foaling stalls should be thoroughly cleaned and disinfected between parturitions. Ideally, the mare's udder, hindquarters and perineum should be cleaned and dried after foaling, and before the foal's introduction to the new stall. Stalls should then be cleaned at least once a day and fresh bedding should be provided. Moreover, the foal's umbilicus should be treated appropriately to reduce the risks of umbilical infections. A 4% chlorhexidine solution has shown to be effective in human neonates and is often used for neonatal foals (Sanchez, 2005).

Prophylactic antimicrobial treatment was recommended for all neonatal foals for a long time. However, a recent study found no differences in occurrence of infectious diseases in neonates prophylactically treated with antimicrobials and those not treated (Wohlfender, Barrelet, Doherr, Straub & Meier, 2009). Improving management practice and hygienic standards seems to suffice, and routine prophylactical antimicrobial use is no longer advised (Dunkel & Johns, 2015).

An adequate colostrum intake is vital, and the foal's IgG levels should be assessed and addressed when necessary. The quantity, quality, volume and timing of colostrum intake are all important factors that should be monitored. A minimum dose of 60 to 90g of IgG, in a good quality colostrum with a total volume of 1 to 1.5L should be taken by the foal in the first 6 hours of life. Measurement of serum IgG should be made between 12 and 18 hours of age. A foal that has consumed adequate quantities of colostrum will have a serum IgG substantially greater than 8 g/L (Liepman *et al*., 2015; Lester & Axon, 2015). FPT is usually defined as IgG<4 g/L. Foals with IgG values between 4 and 8 g/L are considered to have partial FPT (Lester & Axon, 2015).

Despite all preventive methods that can be adopted, early recognition of compromised foals and prompt treatment are the crucial factors to achieve a positive outcome. For that reason, client education in this regard is vital (Dunkel & Corley, 2015).

1.2. The male disadvantage

There is a discipline in human medicine referred to as “gender medicine” that studies the impact of gender on human physiology, pathophysiology and clinical features of diseases (Neubauer, Griesmaier, Ralser & Kiechl-Kohlendorfer, 2012). This concept is based on the perception that males and females are at unequal risk of different diseases. In 1971, a study demonstrated an inherent biological disadvantage of being born male - the so-called “male disadvantage” (Naeye, Burt, Wright, Blanc & Tatter, 1971). In the referred study, the authors found a significantly higher male to female gender ratio for neonatal disorders when compared to the ratio recorded for livebirths, revealing an increased perinatal morbidity in male infants. Since then, several studies have reinforced this concept. First, males are more likely to be born prematurely (Cooperstock & Campbell, 1996; Zeitlin *et al*., 2002), and when born pre-term they are at a higher risk of suffering from neonatal diseases, particularly respiratory distress syndromes (Elsmén, Steen & Hellström-Westas, 2004). Gender disparities have also been reported for sepsis, with males presenting a higher risk in comparison with their female counterparts (Martin, Mannino, Eaton & Moss, 2003; Watson *et al*., 2003; Moss, 2005; Esper *et al*., 2006). Male gender has not only been associated with higher morbidity and mortality rates before discharge, but also with higher morbidity after discharge, resulting in higher rehospitalisation rates for boys (Ambalavanan *et al*., 2011; Kent, Wright & Abdel-Latif, 2012; Neubauer *et al*., 2012). In the long term, male infants also present poorer neurologic outcomes, even after adjustments for severity of illness (Stevenson *et al*., 2000; Kent *et al*., 2012).

Despite all medical advances in neonatal care, the male disadvantage has persisted (Stevenson *et al*., 2000), suggesting that the mere fact of being male is, in itself, an independent risk factor (Neubauer *et al*., 2012). Chromosomal differences could eventually explain this difference, but subsequent events appear to be independent of known X or Y chromosome-related products (Kent *et al*., 2012). Lung immaturity has also been suggested as a cause of poorer outcomes in boys, since lungs seem to be less developed than in girls of the same gestational age (Peacock, Marston, Marlow, Calvert & Greenough, 2012). Differences in the placenta, cardiovascular responses, neural protection and responses in the hypothalamic-pituitary-adrenal axis have also been reported as potential explanations (Kent *et al*., 2012). Yet, the precise mechanisms behind the influence of gender are still unknown.

A study in 2007 investigated the differences in cardiovascular and cerebrovascular responses of male and female sheep foetuses to an acute asphyxial insult. Although no significant differences in outcome were found, males did show impaired haemodynamic adaptation within the normal spectrum, which seems to suggest a differential effect of gender (Bennet *et al*., 2007). If the phenomenon of male disadvantage proves to be true for animals as it is in humans, veterinarians would be provided with a new piece of information for anticipating neonatal morbidities and promoting early treatment. This would be of particular interest in cases of sepsis, in which early intervention is essential for a positive outcome.

1.3. Objectives of this study

Gender has shown to be an influencing factor for the development of neonatal sepsis in human patients, but has not been formally investigated in horses. We hypothesised that male foals would have a higher prevalence of neonatal sepsis when compared to their female counterparts. Based on this hypothesis, the objective of this retrospective study was to compare gender proportions in groups of foals with and without sepsis, admitted to a neonatal intensive care unit in Ireland, over a period of nine years. To the best of our knowledge, this was the first study investigating the influence of gender in the prevalence of equine neonatal sepsis.

II. MATERIALS AND METHODS

2.1. Inclusion criteria

Medical records of all foals admitted to Anglesey Lodge Equine Hospital between January 1, 2008 and July 31, 2016 were reviewed. Foals were eligible for inclusion if they were 28 days old or younger at the time of admission, and if they were diagnosed with sepsis in their medical records. Foals were excluded from the study if their gender was not reported in their record.

A diagnosis of sepsis was achieved by close examination of the foal, performed by a specialist of internal medicine and critical care. Every foal underwent a thorough physical examination and had blood drawn out for complete haematology and biochemistry. Complementary exams such as ultrasound and radiography were performed when found useful by the clinician. Each case was assessed individually and a diagnosis was made based on a strong clinical suspicion of sepsis and/or a positive blood culture. Neither the sepsis score or the modified sepsis score were used to categorise the animals in this study.

2.2. Signalment

Age, date of birth, gender, short-term outcome and blood culture results, when applicable, were collected for each foal. Age was recorded in days. Short-term outcome was defined as survival to hospital discharge. Foals alive at the time of discharge were considered survivors. Foals that died or were subjected to euthanasia were considered nonsurvivors.

2.3. Control group

Foals admitted to the hospital, aged 28 days old or younger, that were not diagnosed with sepsis in their medical records and that had their gender recorded, were included in the study as a control group. Age, date of birth, gender and short-term outcome were collected for each foal. The diagnoses reported on the medical records were also collected. In cases where foals presented more than one medical condition, diagnosis was recorded according to the imminent life-threatening condition (e.g. a foal with a contracted joint and a meconium impaction was recorded as presenting “meconium impaction”).

2.4. Data analysis

Data analysis was performed using R statistical software, version 3.32, for Windows. Descriptive statistical analyses including mean and standard deviation were used to summarise the data related to the foals’ age. Age was tested for normal distribution with the Shapiro-Wilk test. Mean age was compared between groups using the Mann-Whitney *U* test. Comparison of categorical data was made with the Pearson’s Chi-squared test and Fisher’s exact test. A cut-off for statistical relevance was made at a p-value of 0.05.

III. RESULTS

3.1. Clinical cases

A total of 801 medical records were reviewed, of which 248 were excluded for being older than 28 days or by not having their gender reported in their medical records. Of the remaining 553 foals, 85 were diagnosed with sepsis in their medical records, therefore meeting the inclusion criteria previously set.

3.2. Control group

Of the 553 foals, 468 foals were not diagnosed with sepsis and were used as the control population. This resulted in a total of 15.4% (85 foals) diagnosed with sepsis and 84.6% (468 foals) diagnosed without sepsis, as shown in Table 6. Sepsis was the third most common diagnosis (15.4%), preceded only by perinatal asphyxia syndrome (17.9%) and diarrhoea/enteritis (23.7%). Together, these three comprised 57% of the diagnoses.

Table 6: Number of foals included in the sepsis and control groups, and in each gender category.

	n (%)	female	male
Sepsis	85 (15.4)	25	60
Control	468 (84.6)	180	288
Congenital, non-infectious			
Hernia	5 (0.9)	0	5
Limb contracture	19 (3.4)	6	13
Other	14 (2.5)	4	10
Acquired, non-infectious			
Perinatal asphyxia syndrome	99 (17.9)	36	63
Trauma	8 (1.4)	2	6
Acute renal failure	5 (0.9)	3	2
Meconium impaction	16 (2.9)	6	10
Intestinal volvulus	2 (0.4)	2	0
Bladder rupture	13 (2.4)	2	11
Eye injury	1 (0.2)	1	0
Neonatal isoerythrolysis	9 (1.6)	4	5
Colic	17 (3.1)	3	14
Weakness	10 (1.8)	3	7
Other	17 (3.1)	7	10
Other, non-infectious			
Complications at birth	34 (6.1)	17	17
High-risk pregnancy	34 (6.1)	19	15
Focal infection			
Diarrhoea/Enteritis	131 (23.7)	53	78
Focal limb infection	24 (4.3)	10	14
Cellulitis	3 (0.5)	1	2
Rhodococcus equi	1 (0.2)	0	1
Pneumonia	5 (0.9)	1	4
Umbilical infection	1 (0.2)	0	1

3.3. Signalment

Table 7 shows the data collected for the sepsis and control groups. Variables were tested for association with group categorisation, and p-values are shown for each parameter. The analyses are described in more detail in the following topics.

Table 7: Gender, age, year and season of birth and short-term outcome distribution among the 553 foals considered as the base population.

	Category			
	Sepsis (%)	**Control (%)**	**Total (%)**	**p-value**
Total	85	468	553	
Gender				
Male	60 (70.6)	288 (61.5)	348 (62.9)	
Female	25 (29.4)	180 (38.5)	205 (37.1)	0.112[a]
Male:female ratio	2.4:1	1.6:1		0.142[b]
Age (days)				
<7	76 (89.4)	372 (79.5)	448 (81)	
8-14	7 (8.2)	55 (11.8)	62 (11.2)	0.101[a]
15-21	0 (0)	22 (4.7)	22 (4)	0.087[b]
22-28	2 (2.4)	19 (4.1)	21 (3.8)	
Year of birth				
2008	15 (17.6)	70 (15)	85 (15.4)	
2009	4 (4.7)	40 (8.5)	44 (8)	
2010	7 (8.2)	60 (12.8)	67 (12.1)	
2011	10 (11.8)	49 (10.5)	59 (10.7)	0.270[a,1]
2012	5 (5.9)	51 (10.9)	56 (10.1)	0.285[b,1]
2013	8 (9.4)	42 (9)	50 (9)	
2014	16 (18.8)	50 (10.7)	66 (11.9)	
2015	9 (10.6)	61 (13)	70 (12.7)	
2016	11 (12.9)	45 (9.6)	56 (10.1)	
Season of birth				
Winter	16 (18.8)	103 (22)	119 (21.5)	
Spring	61 (71.8)	346 (73.9)	407 (73.6)	0.150[a]
Summer	8 (9.4)	18 (3.8)	26 (4.7)	0.162[b]
Fall	0 (0)	1 (0.2)	1 (0.2)	
Short-term outcome				
Nonsurvivors	37 (43.5)	101 (21.6)	138 (25)	<0.01[a,2]
Survivors	40 (47.1)	318 (67.9)	358 (64.7)	<0.01[b,2]
Unknown	8 (9.4)	49 (10.5)	57 (10.3)	

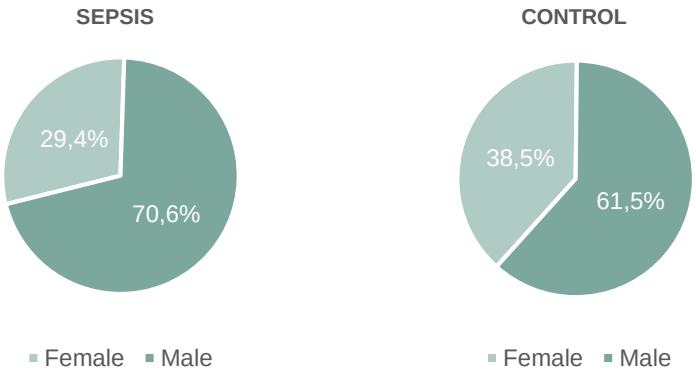
[a]p-value for Pearson's Chi-squared test. [b]p-value for Fishers' exact test.
[1]Statistical analysis was performed considering three groups of years (2008-2010; 2011-2013; 2014-2016).
[2]Statistical analysis was performed after exclusion of the cases in which outcome could not be recalled.

3.3.1. Gender distribution

Of the 85 foals with sepsis, 60 (70.6%) were male and 25 (29.4%) were female, with a male:female ratio of 2.4:1. Of the 468 foals included in the control group, 288 (61.5%) were male and 180 (38.5%) were female, with a male:female ratio of 1.6:1. This is illustrated in Figure 3.

Despite an apparently higher percentage of males in the sepsis group when compared to the control group (70.6% *vs*. 61.5%), analysis of gender distribution showed no significant differences between foals with and without sepsis (Table 7).

Figure 3: Graphic representation of gender distribution in each group.



3.3.2. Age distribution

Age distribution was tested for normality and was found to be not normal ($p<0.001$). Mean age was then compared between groups using a non-parametric test, and showed to be significantly lower in the sepsis group ($p=0.046$).

Table 8: Age range, mean, standard deviation and coefficient of variation in each group.

Group	n	Range	Mean	s.d.	CV
Sepsis	85	0 - 28	3.13	4.78	1.53
Control	468	0 - 28	4.33	6.60	1.53

Age was also analysed as a categorical variable, and was divided in four classes of age: 0 to 7 days old; 8 to 14 days old; 15 to 21 days old; 22 to 28 days old. Figure 4 shows that most foals (81%) were included in the class from 0 to 7 days old. In the sepsis group, 89.4% were 7 days old or younger, 8.2% were 8 to 14 days old and 2.4% were 22 to 28 days old, with no foals being 15 to 21 days old. In the control group, 79.5% were 7 days old or younger, 11.8% were 8 to 14 days old, 4.7% were 15 to 21 days old and 4.1% were 22 to 28 days old. Analysis of age distribution by classes of age between groups showed no significant differences, with p-values of 0.101 for Pearson's Chi-squared test and 0.087 for Fisher's exact test.

Figure 4: Distribution of foals by classes of age in the sepsis and control groups.

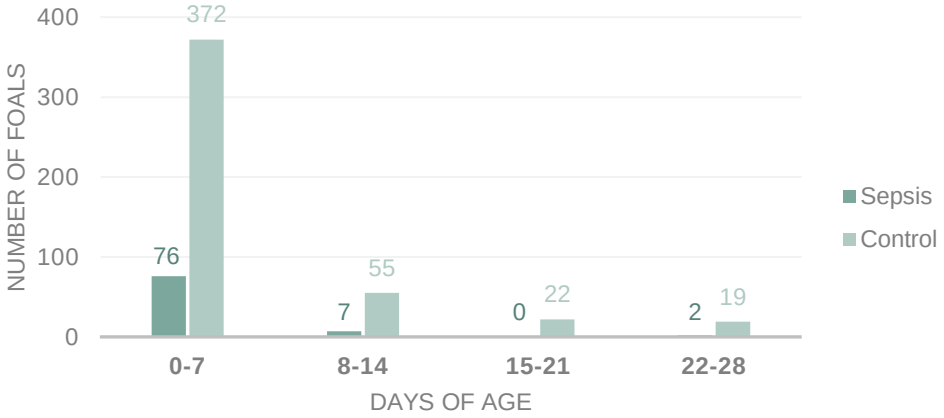


Figure 5: Distribution of foals by age and gender within the sepsis group.

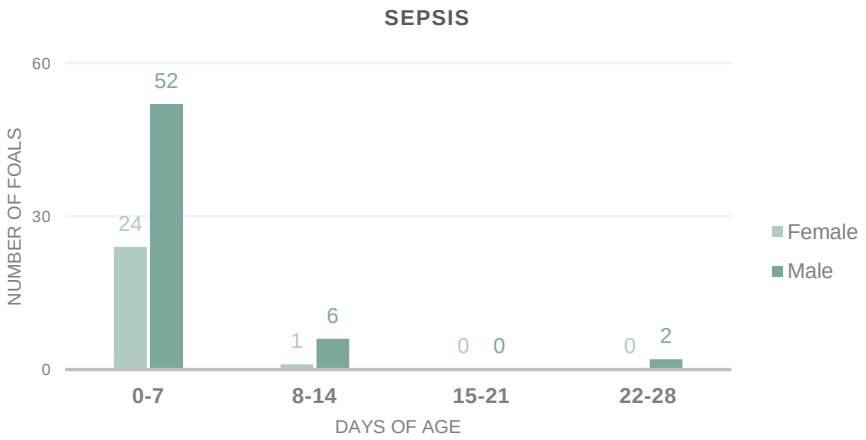
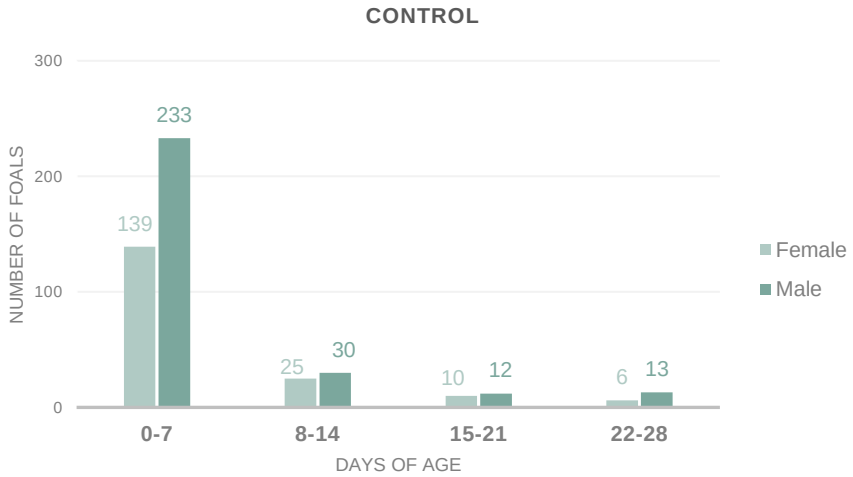


Figure 6: Distribution of foals by age and gender within the control group.



Figures 5 and 6 show the distribution of foals by age and gender within the two groups. In the sepsis group, 96% of the fillies were 7 days old or younger. There was only one female (4%) older than 7 days. As for the males, 86.7% were 7 days old or less, with eight colts (13.3%) being older than 7 days. In the control group, the percentages of foals older than 7 days were 22.8% for the fillies and 19.1% for the colts.

Analysis of gender distribution by classes of age was made for both sepsis and control groups. For the sepsis group, Person's Chi-squared test could not be used because expected frequencies for some of the cells were less than 5. p-value for Fisher's exact test was 0.696. In the control group, both Pearson's Chi-squared test and Fisher's exact test were used, with p-values of 0.539 and 0.537, respectively. There was no association between gender and class of age in both sepsis and control groups.

3.3.3. Birth distribution

3.3.3.1. Year of birth

Table 7 shows the number of foals born per group in each year. 2008 was the year when more births were recorded, while 2009 was the year with less births recorded. Overall, the number of foals born per year ranged from 44 to 85, with a mean value of 61 births per year.

The number of foals with sepsis per year was the highest in 2014 and the lowest in 2009. It ranged from 4 to 16 foals with sepsis, with a mean value of 9 foals with sepsis per year.

Prevalence of sepsis (*[number of foals with sepsis in a given year/number of foals born that year]×100*) was calculated for each year. Values obtained were 17.6% for 2008, 9.1% for 2009, 10.4% for 2010, 16.9% for 2011, 8.9% for 2012, 16% for 2013, 24.2% for 2014, 12.9% for 2015 and 19.6% for 2016. Overall prevalence was 15.4%.

Year of birth was also analysed as a categorical variable, and was divided in three classes: 2008-2010, 2011-2013 and 2014-2016. Analysis showed no significant differences between groups (Table 9).

Table 9: Comparison between group and year of birth in the total population.

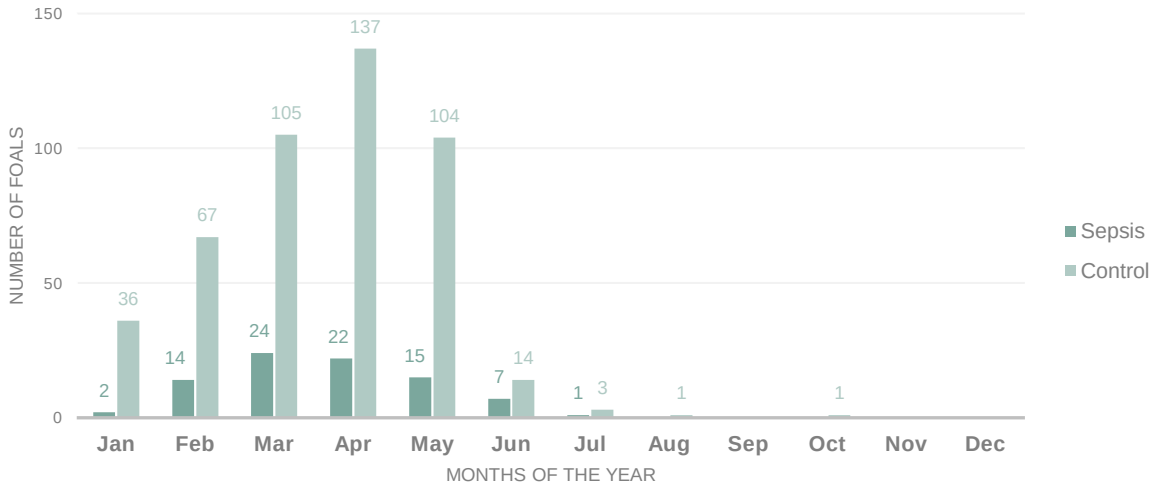
Group	2008-2010	2011-2013	2014-2016
Sepsis	26	23	36
Control	170	142	156

p-value was 0.270 for Pearson's Chi-squared test and 0.285 for Fisher's exact test. No association between groups and classes of year was found.

3.3.3.2. Month of birth

Figure 7 shows the distribution of births throughout the year. Overall, April was the month when more births occurred, with a total of 159 births. When analysing the groups separately, March was the month when more births occurred in the sepsis group, while April was the month when more births occurred in the control group. To facilitate statistical analysis, months were grouped by seasons of the year. Analysis of season of birth showed no significant differences between foals with and without sepsis (Table 7).

Figure 7: Birth distribution of hospitalised foals in each month of their respective year (with vs without sepsis)



Further analysis regarding season of birth and gender was performed.

Figure 8 shows the distribution of births by gender category. Birth distribution showed no significant difference between males and females (Table 10).

Figure 8: Birth distribution of hospitalised foals in each month of their respective year (gender differences).

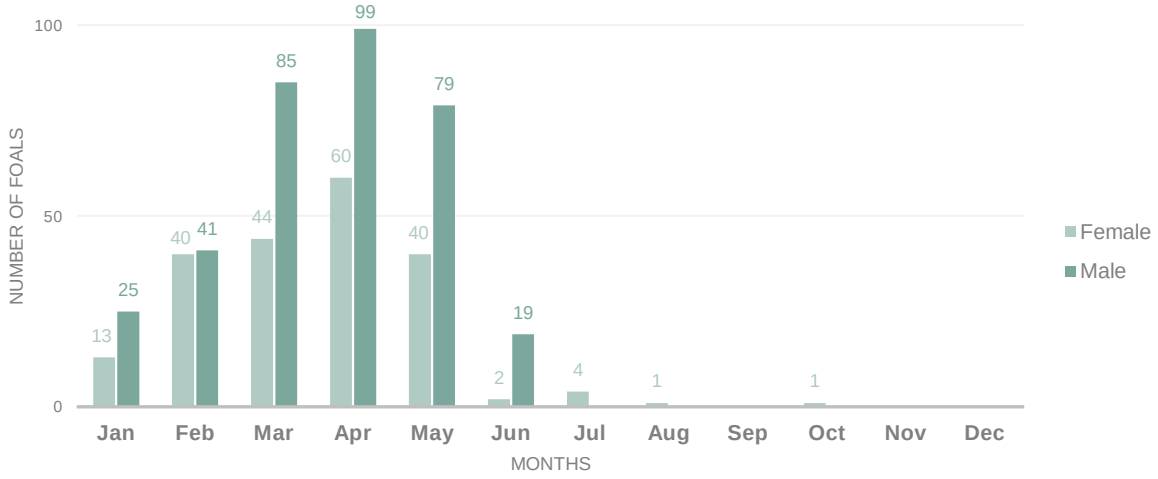


Table 10: Comparison between gender and season of birth in the total population.

Gender	Winter	Spring	Summer	Fall
Female	53	144	7	1
Male	66	263	19	0

p-value was 0.103 for Pearson's Chi-squared test and 0.083 for Fisher's exact test. No association between gender and season of birth was found.

The distribution of births within each group and according to gender and was also analysed. It was observed that in the sepsis group (Figure 9), April was the month when more males were born, while March was the month when more females where born. In the control group (Figure 10), April was the month when more males and females were born.

Figure 9: Birth distribution of hospitalised foals in each month of their respective year (foals with sepsis).

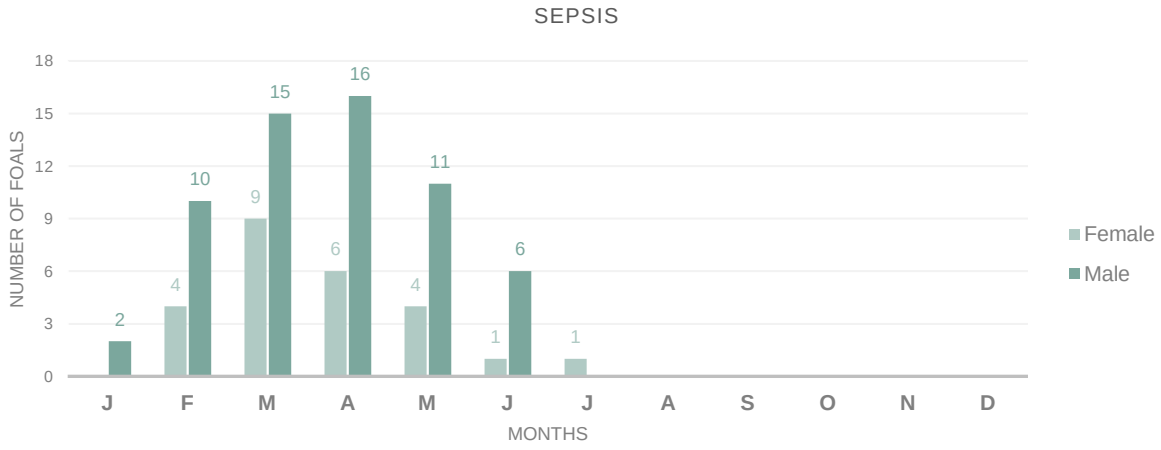
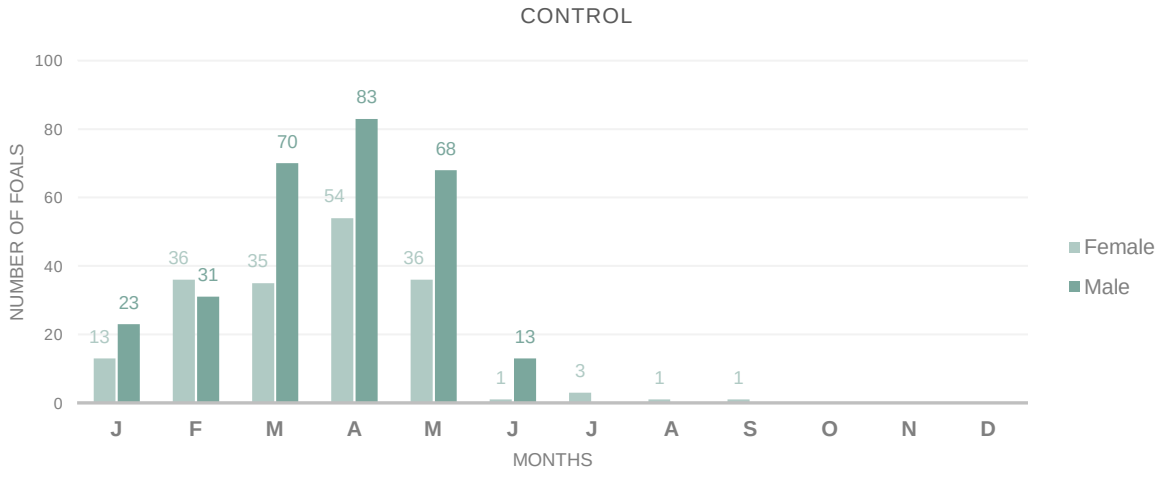


Figure 10: Birth distribution of hospitalised foals in each month of their respective year (foals without sepsis).



The association between gender and season of birth, for both the sepsis and the control group, was investigated. For the sepsis group, Person's Chi-squared test could not be used since some of the expected frequencies were less than 5. Fisher's exact test was used and showed no significant differences between gender and season of birth (Table 11). For the control group (Table 12), Pearson's Chi-squared test and Fisher's exact test were used and showed no significant differences between gender and season of birth.

Table 11: Comparison between gender and season of birth in the sepsis group.

Sepsis group				
Gender	Winter	Spring	Summer	Fall
Female	4	19	2	0
Male	12	42	6	0

Pearson's Chi-squared test was not used because some of the expected frequencies were less than 5. p-value was 0.927 for Fisher's exact test. No association between gender and season of birth was found in the sepsis group.

Table 12: Comparison between gender and season of birth in the control group.

Control group				
Gender	Winter	Spring	Summer	Fall
Female	49	125	5	1
Male	54	221	13	0

p-value was 0.058 for Pearson's Chi-squared test and 0.076 for Fisher's exact test. No association between gender and season of birth group was found in the control group.

3.3.4. Short-term outcome

Of the 553 foals, 57 did not have their outcome recorded. Of the 57 foals, 8 belonged to the sepsis group and 49 belonged to the control group. All calculations were made after exclusion of the cases where outcome could not be recalled.

The overall mortality rate was 27.8%, with a total of 138 nonsurvivors in 496 foals. Sepsis was responsible for 26.8% of deaths, followed by perinatal asphyxia syndrome with 20.3% and complications at birth with 13%.

Table 13: Number and percentage of nonsurvivors and survivors, according to the different diagnoses.

	Nonsurvivors (%)	Survivors (%)	Total
Sepsis			
Sepsis	37 (26.8)	40 (11.2)	77
Control			
Congenital, non-infectious			
Hernia	1 (0.7)	4 (1.1)	5
Limb contracture	4 (2.9)	8 (2.2)	12
Other	8 (5.8)	5 (1.4)	13
Acquired, non-infectious			
Perinatal asphyxia syndrome	28 (20.3)	64 (17.9)	92
Trauma	3 (2.2)	3 (0.8)	6
Acute renal failure	2 (1.4)	3 (0.8)	5
Meconium impaction	0 (0)	16 (4.5)	0
Intestinal volvulus	2 (1.4)	0 (0)	2
Bladder rupture	3 (2.2)	8 (2.2)	11
Eye injury	0 (0)	1 (0.3)	1
Neonatal isoerythrolysis	2 (1.4)	7 (2)	9
Colic	3 (2.2)	10 (2.8)	13
Weakness	1 (0.7)	8 (2.2)	9
Other	8 (5.8)	7 (2)	15
Other, non-infectious			
Complications at birth	18 (13)	11 (3.1)	29
High-risk pregnancy	2 (1.4)	32 (8.9)	34
Focal infection			
Diarrhoea/Enteritis	11 (7.9)	106 (29.6)	117
Focal limb infection	3 (2.2)	18 (5)	21
Cellulitis	0 (0)	3 (0.8)	3
Rhodococcus equi	0 (0)	1 (0.3)	1
Pneumonia	2 (1.4)	2 (0.6)	4
Umbilical infection	0 (0)	1 (0.3)	1
TOTAL	138	358	496

Survival rate in foals with sepsis was 51.9% (40/77). Analysis of short-term outcome between foals with and without sepsis showed that survival rate was significantly higher for foals without sepsis when compared to foals with sepsis (75.9% *vs*. 51.9%), with p-values <0.01 for both Pearson's Chi-squared test and Fisher's exact test (Table 7).

Further analysis regarding gender and outcome was performed (Table 14). Despite an apparently higher percentage of male nonsurvivors when compared to female nonsurvivors (29.4% *vs*. 25.3%), differences were not considered significant

Table 14: Statistical comparison between gender and outcome.

Gender	Nonsurvivors	Survivors
Female	47	139
Male	91	219

p-value was 0.352 for Pearson's Chi-squared test and 0.352 for Fisher's exact test. No association between gender and outcome was found.

Additional analyses were performed comparing group and outcome within each gender category. The percentage of survivors was not significantly different in females with sepsis when compared to females without sepsis (68.2% *vs*. 75.6%, p>0.05), as shown in Table 15. However, the percentage of survivors was significantly lower in males with sepsis when compared to males without sepsis (45.5% *vs*. 76.1%, p<0.01), as shown in Table 16.

Table 15: Statistical comparison of females with and without sepsis and outcome.

Females

Group	Nonsurvivors	Survivors
Sepsis	7	15
Control	40	124

p-value was 0.451 for Pearson's Chi-squared test and 0.442 for Fisher's exact test. No association between groups and outcome was found in the female category.

Table 16: Statistical comparison of males with and without sepsis and outcome.

Males

Group	Nonsurvivors	Survivors
Sepsis	30	25
Control	61	194

p-value was <0.01 for both Pearson's Chi-squared test and Fisher's exact test. An association between groups and outcome was found in the male category.

3.3.5. Blood culture results

A total of 27 blood cultures were performed, of which 13 were positive (48.1%). Of the 14 foals with unconfirmed blood infection, 7 foals were diagnosed with sepsis in their medical records. A total of 21 bacteria were isolated from 13 cultures. Most blood cultures (61.5%) showed growth of a single bacterium, while 38.5% showed mixed infection. Of the cultures with single bacterial growths, 50% were Gram-negative and 50% Gram-positive. Of the mixed cultures, 60% showed at least one Gram-positive and one Gram-negative bacteria, while the remaining 40% showed only Gram-positive bacteria.

The mean number of isolates per foal was 1.6 and the median was 1 isolate. The type and frequency of isolated pathogens are shown in Table 17. Overall, 47.6% of the bacteria were gram-negative and 52.4% were gram-positive. *E. coli* was the most frequently isolated bacterium, accounting for 23.8% of all isolates. The five cases of mixed infections were as follows: *Bacilllus spp*. and α-haemolytic *streptococci*; *E. coli* and *Bacillus spp.*; *E.coli*, one unidentified coliform and α-haemolytic *streptococci*; *Actinobacilus equuli*, one unidentified coliform, α-haemolytic *streptococci* and *Fusobacterium spp.*; and *Staphilococcus spp.* and α-haemolytic *streptococci*.

Table 17: Results of the 21 microbiological isolates from the 13 positive blood cultures from foals with sepsis.

Isolates	Total (%)
Gram-negative	**10**
Coliforms	
Escherichia coli	5 (23.8)
Not identified	2 (9.5)
Actinobacillus equuli	1 (4.8)
Serratia marescences	1 (4.8)
Fusobacterium spp.	1 (4.8)
Gram-positive	**11**
Streptococcus spp.	
β-haemolytic *streptococci*	1 (4.8)
α-haemolytic *streptococci*	4 (19)
Staphylococcus spp.	1 (4.8)
Enterococcus spp.	1 (4.8)
Bacillus spp.	2 (9.5)
Clostridium spp.	
Clostridium perfringens	1 (4.8)
Not identified	1 (4.8)

Viraemia by EHV-1 was suspected in 4 foals, one of which had a positive blood culture for *E. coli*. However, records showed no information about confirmation of these diagnoses, and therefore these results were not included in the statistical analysis.

IV. DISCUSSION

Sepsis is a complex and life-threatening pathophysiological event that inevitably leads to death, unless promptly addressed. Its diagnosis remains problematic, not only due to its unspecific clinical signs but also due to the lack of uniformity in the criteria used to define sepsis in different clinical studies. Adapting criteria may not be wrong in itself, and may even be required for horses in different clinical settings. However, the lack of standardisation results in slightly different populations for comparison purposes, making it challenging to generalise information from single clinical studies.

In the present study, foals were diagnosed with sepsis based on the medical records obtained. This classification was made according to an internal medicine specialist attending the cases, who was also responsible for the NICU of this hospital (K.C.). Hence, in this study, a diagnosis of sepsis was achieved based on a strong clinical suspicion and/or a positive blood culture. The sepsis score was not used to categorise the animals, as evidence suggests it is not suitable to define sepsis unless first validated in the hospital due to its poor sensitivity, specificity and negative predictive values (Corley & Furr, 2003). Although studies should have well defined inclusion criteria, and the use of a scoring system would have been desirable for comparison purposes, incorrectly predicting foals to not have sepsis may result in inadequate treatment and reduced chance of a favourable outcome, while incorrectly predicting foals to have sepsis may lead to resource wastage and possibly unwarranted euthanasia on economic grounds. The experience of the attending clinician as well as the temporal clinical limitations lead to a definition of sepsis based on an overall interpretation of clinical signs and laboratory findings rather than a point by point score.

In our study, 15.4% of the considered population was diagnosed with sepsis. The prevalence of sepsis varies between studies: Hoffman *et al*. (1992) documented a percentage of 49% septic foals; Peek *et al*. (2006) indicated a value of 67.6%; Armengou *et al*. (2013) reported 51%; Dembek *et al*. (2014) reported 40% for the retrospective part of their study and 38% for the prospective part. Our study showed a lower percentage than the referred studies, and numerous reasons may be accounted responsible for this. The allocation of the hospital is in a well-known area of horseracing, and a great amount of the foals admitted are usually Thoroughbred foals from local stud farms. The commercial nature and experience in breeding of these studs allow them to have refined management practices, including excellent *peri* and *post partum* monitoring which may prevent infections and be partially responsible for a lower prevalence of sepsis in this population of foals. On the other hand, genetic factors may also play a role: genetic mutations have been previously associated with enhanced susceptibility to infection in horses - a lethal genetic disorder termed severe combined immunodeficiency (SCID) has been described in Arabian foals, and refers to individuals that are incapable of generating antigen-specific immune responses (Perryman, 2004). In humans, host genetic factors are also known to influence the susceptibility to infectious diseases, not only through

evident mutations, but also through subtler and complex genetic variations (Kwiatkowski, 2000). It is possible that these subtle variations also happen in foals, with certain populations being less susceptible to infection than others. This could eventually explain the lower prevalence of sepsis in our study population.

We have also identified other potential reasons inherent to the study design: (1) The majority of foals admitted to the hospital were referred cases, and it is possible that some animals with sepsis may have died before ever being admitted to the hospital. Also, (2) some cases were excluded because foals did not have their gender reported in the records; this could have resulted in some foals with sepsis being excluded, falsely decreasing the prevalence of the disease. Finally, (3) comparisons among clinical studies are of limited value when the criteria for admission varies among studies. This is particularly relevant for sepsis, where the ultimate decision in diagnosing a horse is clinician dependent and empirical, and may result in slightly different populations. Still, previous clinical studies have had lower prevalence rates than the present study. An example is the study by Wofhlender *et al*. (2009), which had a surprisingly low prevalence of 0.1%, with only 1 foal with sepsis in 992 foals considered. Yet, no theories to explain this disparity were proposed.

Galvin and Corley (2010) reported that most foals have at least one infectious condition during the first 12 months of life. Of the foals considered in our study, 45.2% foals presented some form of infection, with infectious processes being the main cause of morbidity in this population. Diarrhoea/enteritis was the most common diagnosis (23.7%). This finding is in accordance with reports by Cohen (1994), that state that diarrhoea is the most reported cause of disease in foals less than 7 days old. However, the results in our study may have been influenced by the way groups were categorised. In cases where foals presented more than one medical condition, diagnosis was recorded according to the imminent life-threatening condition, which means that less severe diseases may have been underrepresented.

The main purpose of our study was to analyse the influence of gender in the prevalence of neonatal sepsis in horses. To our knowledge, no studies have been performed regarding this matter. A previous study by Toth *et al*. (2015) reported an apparently higher percentage of males with sepsis when compared to the percentages of healthy, sick without sepsis and critically ill males. However, such data was not analysed, and we cannot infer if these differences were statistically relevant. We can only assume that the lack of analyses in this, and other clinical studies, are due to widespread opinion that gender is not a relevant factor in the pathophysiology of sepsis in foals. A fact that is well refuted in human studies where boys are known to be more susceptible to septic events (Martin *et al*., 2003; Watson *et al*., 2003; Moss, 2005; Esper *et al*., 2006), and studies have proved that being male is an independent risk factor for sepsis (Neubauer *et al*., 2012). We therefore hypothesised that male foals would have a higher prevalence of neonatal sepsis when compared to their female counterparts. To test this hypothesis, we compared gender distribution between a group of foals with sepsis and

a control group. Despite an apparently higher percentage of males with sepsis (70.5%) when compared to males without sepsis (61.5%), differences were not considered significant. The small number of foals comprised in the sepsis group might have influenced the results, and it is possible that an association would have been detected with a higher number of subjects. Furthermore, due to the retrospective nature of our study, we were analysing a specific population of sick foals, sampled from one geographical area and admitted to a single NICU, which may have introduced a selection bias in our data. Also, since males tend to be economically more valuable than females, it is possible that they were overrepresented in the control group, as owners may be more likely to invest in male rather than female foals. Lastly, the control group was composed of foals that we cannot assure truly represent the background population in which the foals with sepsis were born. Ideally, this study should have included a bigger number of foals with sepsis, with a control group originated from the same population, so that proportions of males and females could be compared and a reliable result could have been obtained. In a clinical setting this is highly unlikely, and our approach was the best within our limitations.

Age distribution was also analysed. It is known that the risk of disease is usually greater among foals in the youngest age group (Cohen, 1994). This trend was noticeable in our study, with 81% of all sick foals being less than 7 days old. Yet, mean age was significantly lower ($p=0.04$) in the foals with sepsis comparing to the foals without sepsis (3.13 days *vs.* 4.33 days). This may reflect the early onset and severity of sepsis, with foals with sepsis being more quickly admitted to the hospital, and therefore being younger at admission. However, when age was compared as a categorical variable, no significant differences were found between groups. Gender and age were also compared within each group, but no associations were found.

Analyses of birth distribution were made regarding year of birth. 2008 was the year with more births recorded (n=85), and 2014 was the year with the highest number of foals with sepsis (n=16). Statistical analysis was performed, but distribution of births in classes of years showed no association with group categorisation or gender.

Regarding the time of year when the foal was born, the results show that April was the month when more foals were born (n=157), with Spring being the season of the year with more births (n=407). This was an expected finding that illustrates the breeding season for horses in the northern hemisphere. When foals with and without sepsis were separately analysed for association with seasons of birth, no associations were found. Gender and season of birth within each group were also analysed, but showed no association.

Sepsis was the disease responsible for the highest percentage of deaths (26.8%) in our study, which is in accordance with previous papers that describe sepsis as a major cause of death in foals (Cohen, 1994). Nevertheless, we must take into consideration that mortality rate in our study comprises foals who were euthanised, and that reasons to subject a foal to euthanasia include not only a guarded prognosis, but also financial or humane motives.

In our study, a survival rate of approximately 51.9% was obtained for foals with sepsis. Survival rates in neonatal foals with sepsis have been documented to vary widely, ranging from as low as 10% (Hoffman *et al*., 1992) to as high as 71% (Raisis, Hodgson & Hodgson, 1996; Toth *et al*., 2014). Yet, most studies indicate a survival rate between 46 and 65% (Wong & Wilkins, 2015). The survival rate in our study falls within the interval considered.

Foals with sepsis in our study showed a significantly higher mortality (48.1%) when compared to the foals without sepsis (24.1%) ($p<0.01$). A diagnosis of sepsis has been significantly associated with nonsurvival in previous studies (Hoffman *et al*., 1992), and our study supports this premise.

Further analyses regarding gender and outcome were performed, and despite an apparently higher percentage of male nonsurvivors when compared to female nonsurvivors (29.4% *vs*. 25.3%), differences were not considered significant. This is in accordance with a study by Furr *et al*. (1997), in which the authors found no association between outcome and gender in two populations of critically ill neonates.

In the present study, statistical analyses showed that foals with sepsis had higher mortality rates. Since gender was not associated with either group categorisation or outcome, it could have been assumed that mortality rates would be higher for foals with sepsis in both gender categories. However, when each gender category was analysed separately, only males with sepsis showed a significantly higher mortality rate ($p<0.01$); no significant difference was found between females with or without sepsis. This result suggests that, in a universe of foals with sepsis, males have higher mortality when compared to females, indicating that gender may have an influence in the clinical evolution of a septic process. This finding is in accordance with other studies from human medicine, which correlate male gender with higher mortality rates before discharge (Stevenson *et al*., 2000; Kent at al., 2012).

As far as blood cultures are concerned, it was possible to isolate the microorganisms that initiated the septic processes in 13 foals, although only 27 were tested. Most cultures showed growth of a single bacterium, yet 38.5% showed mixed infections. A total of 21 bacteria were isolated, with Gram-positive microorganisms being more frequent (52.4%). Although most organisms obtained from blood cultures of neonatal foals are usually Gram-negative enterobacteria, the percentage of Gram-positive isolates has increased significantly over the years (Dunkel & Corley, 2015). This may explain the higher percentage of Gram-positive bacteria isolated in our study. Still, only a few number of microorganisms were identified in this population, which may not be representative of the bacteria that affected these foals.

Escherichia coli was the most frequently isolated microorganism in our study, accounting for 23.8% of the isolates. Because regional differences in prevalence of bacteria occur (Theelen *et al.*, 2014), the frequencies observed may be exclusive to our hospital. However, *E. coli* has also been identified as the most frequently isolated bacterium in foals with sepsis in other

studies (Corley *et al.*, 2007; Russell *et al.*, 2008; Theelen *et al.*, 2014), suggesting that the results of our study may be applicable to other geographic locations.

Differences in gender and survival rates between Gram-positive and Gram-negative bacteria were beyond the scope of our study, and the small number of positive blood cultures impeded further investigation. To date, studies showed no difference in survival rates between Gram-positive and Gram-negative bacteria (Stewart *et al.*, 2002; Corley *et al.*, 2007).

The present study had some limitations, and the retrospective nature of this study was the main limitation encountered. We were dependent on the accuracy and completeness of medical records for our data, which wasn't always possible and led to the exclusion of several medical records. Some information may have been inadvertently not recorded because foals are often admitted in an emergency situation. The potential bias this may have introduced into the results cannot be directly determined, and the results presented and discussed here will need to be tested in a large prospective study.

To our knowledge this was the first time that an association between outcome, group and gender categorisation was ever made for equine neonatal sepsis, and the results reveal numerous new avenues in the study of neonatal disorders. Recognising male gender as an indicator of poor prognostic may help predicting the outcome of foals with sepsis and assist owners in the decision-making process regarding treatment, allowing resources to be better spent. More importantly, it may help increasing survival rates, providing special caution is taken during gestation, parturition and *post-partum* periods of male neonates.

It is now important to understand the exact cause for this disparity. It is known that genetic mutations influence foals' susceptibility to infection (Perryman, 2004), but the eventual association of subtle genetic variations with sepsis warrants further investigation. Differences in the gestational development of males and females (Peacock *et al.*, 2012) and neuroendocrine disparities (Kent *et al.*, 2012) were associated with different susceptibility to infection in human neonates, and may reveal of interest in foals. Identifying the source may help addressing the causative factors with more precision, ultimately contributing to the prevention of the disease.

V. CONCLUSION

The intensive care of critically ill neonates is one of the most challenging tasks in equine medicine, with sepsis remaining the most frequent cause of death in foals (Palmer, 2014). Survival rates for foals with sepsis have been reported to vary among studies (Wong & Wilkins, 2015), but mortality is usually higher for animals with sepsis when compared to other sick animals without sepsis (Hoffman *et al*., 1992). In our study, sepsis proved to be not only the disease responsible for the highest percentage of deaths (26.8%), but also one of the diseases with higher mortality rates (48.1%). Therefore, being able to identify populations of foals at an increased risk of developing sepsis, as well as their prognosis for survival, is vital to help owners making informed decisions regarding treatment of critically ill foals.

Although often dismissed by clinicians, gender may influence the pathophysiology of diseases; a fact well supported by human studies where boys are reported to be at a higher risk of neonatal morbidities, and particularly predisposed to septic processes (Martin *et al.*, 2003; Watson *et al*., 2003; Moss, 2005; Esper *et al*., 2006). To the best of our knowledge, this had not been formally investigated in foals; thus, we proposed to investigate the influence of gender in the prevalence of neonatal sepsis in horses.

Our study compared gender proportions between groups of foals with and without sepsis, admitted to a neonatal intensive care unit in Ireland from 2008 to 2016. Despite an apparently higher percentage of males in the sepsis group when compared to the control group (70.6% *vs*. 61.5%), analysis of gender distribution showed no significant differences ($p>0.05$). However, when comparing the outcome of animals with and without sepsis within each gender category we found a significantly higher mortality rate in males with sepsis compared to males without sepsis (54.5% *vs*. 23.9%, $p<0.01$), while no significant differences were found between females with and without sepsis (31.8% *vs*. 24.4%, $p>0.05$). This was the first time that an association between outcome, group and gender categorisation was ever made for equine neonatal sepsis, and it showed that male gender influences the clinical evolution of septic processes.

Further studies investigating the extent to which male gender influences the clinical evolution of a septic process are now warranted. If the phenomenon of “male-disadvantage” proves to be true for horses as it is in humans, it is possible that male foals might present higher morbidity rates after discharge, with eventually higher rehospitalisation rates and poorer prognosis in the long-term. In those circumstances, males with sepsis may reveal less profitable, not only in the perinatal period but eventually in later periods in life, which might have a huge economic impact in the industry. On the other hand, recognising the prognostic value of being male may increase survival rates, providing special caution is taken during gestation, parturition and *post-partum* periods of male neonates. It may also help predicting the outcome of these foals and assist owners in the decision-making process regarding treatment, allowing resources to be better spent.

The precise mechanisms behind the influence of gender are still unknown, and thus new lines of research emerge. With the development of comparative genomics, it is possible that genetic polymorphisms that influence the response of horses to infection may be found in the future. Eventual disparities in gestational development of males and females and impaired cardiovascular, cerebrovascular and neuroendocrine responses among genders that could explain a foal's enhanced susceptibility to infection should also be explored. Identifying the cause for the disparity between males and females may lead to the improvement of the host resistance and help with early identification of susceptible animals. In this way, prophylactic or therapeutic interventions can be undertaken, ultimately contributing to the prevention of the disease.

Further investigation regarding gender as a predisposing factor for sepsis are also needed, but these analyses are likely to require larger data set. Although gender distribution showed no significant difference between foals with and without sepsis in our study, it is possible that an association would have been detected with a higher number of subjects. Recognising male gender as a predisposing factor for sepsis would help decreasing its prevalence and would allow for early diagnosis and treatment, ultimately improving outcome. This encourages the analysis of gender predisposition in a larger prospective study.

Altogether, our study shows that gender influences the clinical evolution of septic processes in foals, suggesting that it should be considered a valuable parameter when investigating neonatal disorders in horses.

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